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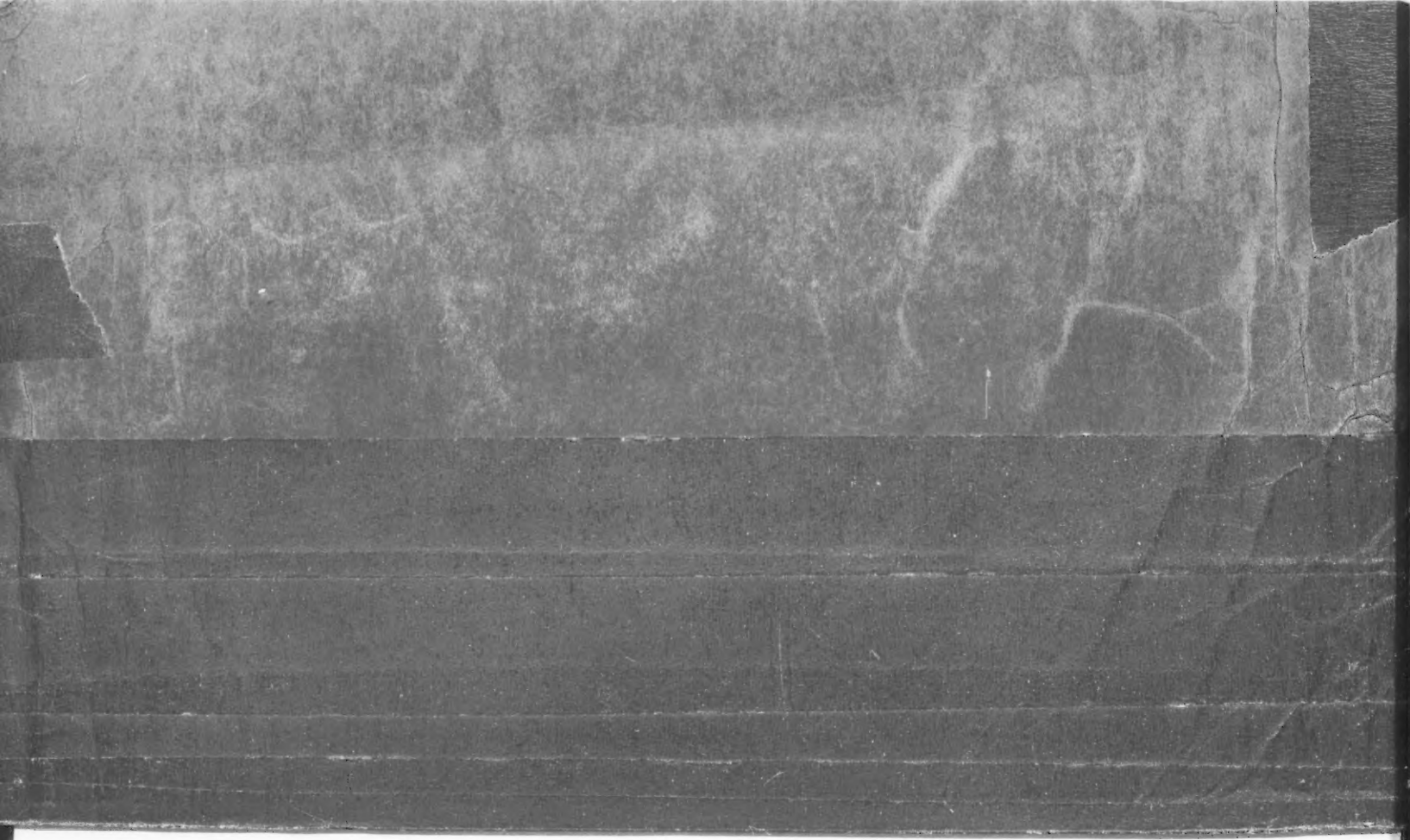

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THE GENETICS OF ALPHA-METHYL-GLUCOSIDE FERMENTATION IN SACCHAROMYCES

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1. INTRODUCTION

It has been established that in Saccharomyces the fermentation of a given sugar can be controlled by several genes. For example, for the fermentation of maltose and raffinose, four and three polymeric genes, respectively, have been demonstrated by Winge and Roberts (1952). For galactose fermentation, three complementary genes are known (Hawthorne, 1956). Since first reporting a single gene controlling alpha-methyl-glucoside fermentation, Lindegren (1949) has published data on the irregular segregations at this locus, attributing to "gene conversion" the ratios deviating from the 2 : 2 expected in tetrads from a hybrid heterozygous for a single gene (Lindegren, 1953; and Lindegren and Lindegren, 1956). The work reported below indicates that a more complex genetic situation exists in our breeding stocks, in that at least five different genes can be demonstrated to be involved in the fermentation of alpha-methyl-glucoside.

2. MATERIALS AND METHODS

The parental haploid strains used in the crosses that follow were either obtained directly from Dr C. C. Lindegren (clones 1426A and 1428) or were derived from cultures originating in his laboratory. With two exceptions, the crosses were made either by pairing haploid cells (Chen, 1950) or by selecting prototrophic diploid clones from a mixture of auxotrophic haploid parents that had been plated on minimal medium (Pomper and Burkholder, 1949). In the two exceptions, crosses 4 and 8, a mass mating of haploid cells was made as described by Lindegren and Lindegren (1943).

The sporulation procedure suggested by Lindegren (1949) was followed. Whenever it was feasible, only linear four-spored asci were dissected (Hawthorne, 1955). Only asci in which all four spores survived were retained for genetic analysis. Since the hybrids were heterozygous for from five to ten genes in addition to the genes controlling alpha-methyl-glucoside fermentation, it was possible to recognise and eliminate from the analysis anomalous asci due to polyploidy (Roman, Hawthorne and Douglas, 1951) or post-meiotic nuclear fusion (Roman and Sands, 1953; Winge and Roberts, 1954).

The tests for the fermentation of alpha-methyl-glucoside were made in Kahn tubes containing 1·5 c.c. of the following medium adjusted to *p*H 6·0 : 1 per cent. peptone, 1 per cent. yeast extract, 1 per cent. alpha-methyl-glucoside, and the indicator bromcresol purple. An alpha-methyl-glucoside fermenter grows vigorously in this medium and causes a change in the colour of the indicator due to acid production. The reliability of this testing procedure has been confirmed by duplicate tests using Durham fermentation tubes.

3. RESULTS

Evidence for the genes MG_1 *and* MG_2

The first indication that more than one locus was involved in alpha-methyl-glucoside fermentation was obtained from the results of crosses (table 1) in which the haploid parents were non-fermenters. In all four cases, the diploid hybrids turned out to be fermenters and the asci obtained from them exhibited the 2 : 2, 1 : 3, and 0 : 4 segregations expected if two loci were involved. The distributions of these ratios among 35 asci, 9 (2 : 2), 21 (1 : 3), and 5 (0 : 4), suggested that the parental haploids differ in two genes, MG_1 and MG_2, both of which are needed for alpha-methyl-glucoside fermentation, and that the three ascus types have the following compositions.

Spore *a*	$MG_1\,MG_2$	(+)	$MG_1\,MG_2$	(+)	$MG_1\,mg_2$	(−)
Spore *b*	$MG_1\,MG_2$	(+)	$MG_1\,mg_2$	(−)	$MG_1\,mg_2$	(−)
Spore *c*	$mg_1\,mg_2$	(−)	$mg_1\,MG_2$	(−)	$mg_1\,MG_2$	(−)
Spore *d*	$mg_1\,mg_2$	(−)	$mg_1\,mg_2$	(−)	$mg_1\,MG_2$	(−)

The genotype of the common parent in the first three crosses, 1011-1*a*, was designated $mg_1\,MG_2$. The other parents, 1001-17*a*, 1001-8*d*, and 1001-14*a*, were on this hypothesis $MG_1\,mg_2$. The negative segregants could be tested to determine if they were of the genotype $MG_1\,mg_2$, $mg_1\,MG_2$, or $mg_1\,mg_2$ by making crosses to the above haploid parents and then inoculating the mixture containing zygotes into the alpha-methyl-glucoside medium. If the zygotes give rise to fermenters, the haploid being tested is of the complementary genotype. In this way, the genotypes of the parents of the fourth cross were found to be $mg_1\,MG_2$ for 133-1*b* and $MG_1\,mg_2$ for 1428.

In a series of crosses made to confirm the zygote test described above, non-fermenter segregants of each of the postulated genotypes were crossed with fermenter haploids. The hybrids in every case were fermenters. The crosses involving the genotypes $mg_1\,MG_2$ and $MG_1\,mg_2$ gave asci with 2 : 2 segregations as expected. Also as expected, the one cross involving the genotype $mg_1\,mg_2$ gave asci with two gene segregations, as shown in table 2.

Evidence for a third alpha-methyl-glucoside gene, MG_3

Two crosses involving non-fermenters of the genotype $MG_1\,mg_2$ and the same fermenter parent, 1003-6*c*, had been expected to give asci with only 2 : 2 segregations, reflecting the segregation of MG_2. Instead only 19 of the 32 asci of crosses 11 and 12 (table 3), gave 2 : 2 segregations and the remaining 13 asci showed 1 : 3 segregations for alpha-methyl-glucoside. These segregations suggested that still another gene was involved in the fermentation of alpha-methyl-glucoside. Additional evidence for the new gene was obtained from the results of a cross in which both haploid parents were non-fermenters. Of the 13 asci from cross 13 (table 3), there were 8 (2 : 2), 4 (1 : 3),

TABLE 1

Segregations for alpha-methyl-glucoside fermentation in asci from hybrids of the genotype $MG_1\ mg_2/mg_1\ MG_2$

Cross	Parents and phenotypes	Hybrid and phenotype	Number of asci segregating as		
			2+ : 2−	1+ : 3−	0+ : 4−*
1	1001-17*a* (−) × 1011-1*a* (−)	133, 135 (+) 136 and 139	3	10	2
2	1001-8*d* (−) × 1011-1*a* (−)	142, 143 (+) 144 and 145	3	8	2
3	1001-14*a* (−) × 1011-1*a* (−)	1015 (+)	2	1	1
4	1428 (−) × 133-1*b* (−)	1017 (+)	1	2	0

* Fermenters (+) : non-fermenters (−).

TABLE 2

Segregations for alpha-methyl-glucoside fermentation in asci from hybrids of the following genotypes: $MG_1\ MG_2/mg_1\ mg_2$ *in cross 5,* $MG_1\ MG_2/mg_1\ MG_2$ *in crosses 6, 7, 8 and 9,* $MG_1\ MG_2/MG_1\ mg_2$ *in cross 10*

Cross	Parents and phenotypes	Hybrid and phenotype	Number of asci segregating as		
			2+ : 2−	1+ : 3−	0+ : 4−*
5	1017-2*d* (+) × 1005-2*d* (−)	1018 (+)	0	7	0
6	1017-2*b* (+) × 1018-4*a* (−)	1019 (+)	3	0	0
7	142-4*c* (+) × 1018-4*a* (−)	1036 (+)	13	0	0
8	1019-1*d* (+) × 136-1*b* (−)	1043 (+)	3	0	0
9	1019-1*c* (+) × 1036-12*c* (−)	1047 (+)	4	0	0
10	1018-6*a* (+) × 1001-16*b* (−)	1033 (+)	6	0	0

* Fermenters (+) : non-fermenters (−).

TABLE 3

Segregations for alpha-methyl-glucoside fermentation in asci from hybrids of the following genotypes: $mg_1\ MG_2\ MG_3/MG_1\ mg_2\ mg_3$ *in crosses 11 and 12,* $MG_1\ mg_2\ MG_3/mg_1\ MG_2\ mg_3$ *in cross 13*

Cross	Parents and phenotypes	Hybrid and phenotype	Number of asci segregating as		
			2+ : 2−	1+ : 3−	0+ : 4−*
11	1003-6*c* (+) × 1001-17*b* (−)	1, 2, 5 (+) 6 and 12	11	9	0
12	1003-6*c* (+) × 1001-14*a* (−)	48, 52 (+) and 84	8	4	0
13	129-3*c* (−) × 143-3*a* (−)	1016 (+)	8	4	1

* Fermenters (+) : non-fermenters (−).

and 1 (0 : 4) segregations. One parent, 143-3*a*, had been tested and found to be of the genotype $mg_1\ MG_2$; the other parent, 129-3*c*, was presumed to be of the genotype $MG_1\ mg_2$. However, the preponderance of 2 : 2 segregations over the 1 : 3 and 0 : 4 segregations, as in the results of crosses 11 and 12, could not be accounted for by any simple scheme involving only two genes MG_1 and MG_2. These results could be explained if it were assumed that there is a third gene, MG_3, which is equivalent to MG_1 in that a combination of the genes MG_2 and MG_3 will enable the yeast to ferment alpha-methyl-glucoside in the absence of MG_1. Thus the excess of 2 : 2 asci over 1 : 3 and 0 : 4 asci from cross 13 would be expected if 129-3*c* were $MG_1\ mg_2\ MG_3$, and 143-3*a* were $mg_1\ MG_2\ mg_3$. Similarly, the 1 : 3 segregations in crosses 11 and 12 can be accounted for if the parents are of genotype

TABLE 4

Segregations for alpha-methyl-glucoside fermentation in asci from hybrids of the following genotypes: $mg_1\ MG_2\ MG_3/mg_1\ MG_2\ mg_3$ *in crosses 14 and 15,* $mg_1\ MG_2\ MG_3/mg_1\ mg_2\ mg_3$ *in cross 16,* $mg_1\ MG_2\ MG_3/mg_1\ mg_2\ MG_3$ *in cross 17,* $MG_1\ MG_2\ mg_3/mg_1\ MG_2\ MG_3$ *in cross 18*

Cross	Parents and phenotypes	Hybrid and phenotype	Number of asci segregating as				
			4+ : 0−	3+ : 1−	2+ : 2−	1+ : 3−	0+ : 4−*
14	1003-6*c* (+) × 145-1*c* (−)	1035 (+)	0	0	21	0	0
15	1035-6*c* (+) × 1036-2*c* (−)	1040 (+)	0	0	11	0	0
16	1003-6*c* (+) × 133-3*a* (−)	1034 (+)	0	0	2	9	1
17	1035-2*b* (+) × 1034-1*d* (−)	1037 (+)	0	0	9	0	0
18	1036-7*a* (+) × 1040-7*b* (+)	1078 (+)	2	9	3	0	0

* Fermenters (+) : non-fermenters (−).

$mg_1\ MG_2\ MG_3$ (1003-6*c*) and $MG_1\ mg_2\ mg_3$ (1001-17*b* and 1001-14*a*). If the three genes segregate independently and show no centromere linkage, a hybrid heterozygous for all three would give 2 : 2, 1 : 3, and 0 : 4 segregations in the ratio of 19 : 16 : 1. The actual segregations obtained for the 45 asci of crosses 11, 12, and 13, 27 (2 : 2), 17 (1 : 3), and 1 (0 : 4), do not differ significantly from the expected frequencies : 23·75 (2 : 2), 20 (1 : 3), and 1·25 (0 : 4).

With this indication of a third alpha-methyl-glucoside gene, MG_3, the crosses reported in the previous section were re-examined. The results are consistent with the interpretation that the parental strains, 1001-8*d*, 1001-14*a*, 1001-17*a*, 1011-1*a*, 1428, and 1005-2*d*, and consequently all segregants carried the recessive gene mg_3.

For further evidence of the correctness of the above explanation, the following crosses were made. The fermenter presumed to be $mg_1\ MG_2\ MG_3$, 1003-6*c*, was crossed to a non-fermenter, 145-1*c*, of genotype $mg_1\ MG_2\ mg_3$, and as expected from a hybrid homozygous for MG_2 and heterozygous for MG_3, only 2 : 2 segregations were observed in the 21 asci from this cross (cross 14, table 4). From another

hybrid of this same genotype, $mg_1\ MG_2\ MG_3/mg_1\ MG_2\ mg_3$, 11 more asci with only 2 : 2 segregations were obtained (cross 15, table 4).

To obtain haploids of the genotype $mg_1\ mg_2\ MG_3$, 1003-6*c* was crossed to a non-fermenter strain of the genotype $mg_1\ mg_2\ mg_3$, as determined by the zygote tests. The 12 asci from cross 16 (table 4) show a distribution of 2 : 2, 1 : 3, and 0 : 4 segregations close to that expected from two independently segregating complementary genes, *i.e.* 2 : 9 : 1 versus 2 : 8 : 2. A confirmation of the zygote tests of the segregants from cross 16 was obtained when a non-fermenter of the predicted genotype $mg_1\ mg_2\ MG_3$ was crossed to a fermenter from cross 14, genotype $mg_1\ MG_2\ MG_3$, to give a hybrid which yielded only the expected 2 : 2 segregations in the nine asci analysed (cross 17, table 4).

A cross of two alpha-methyl-glucoside fermenters was made to substantiate the contention that MG_1 and MG_3 are polymeric genes. One haploid parent, 1036-7*a*, was from cross 7 where 2 : 2 segregations were obtained for MG_1 and the complementary gene, MG_2, was homozygous dominant. Similarly, the other parent, 1040-5*b*, was from cross 15 where 2 : 2 segregations were obtained for MG_3 and again MG_2 was homozygous dominant. The distribution of the segregations, 2 (4 : 0), 9 (3 : 1), and 3 (2 : 2) for the 14 asci (cross 18, table 4) is in accordance with the ratios expected for two independently segregating genes which show little or no centromere linkage.

Evidence for the gene MG_4

When crosses were made to a new fermenter strain, haploid 1426A, received from Dr Lindegren, segregation ratios were observed which indicated that a new gene combination was enabling this haploid to ferment alpha-methyl-glucoside. Haploid 1426A was first crossed to a strain of genotype $mg_1\ MG_2\ mg_3$, 1018-4*a*, previously used as the non-fermenter parent in crosses 6 and 7. The segregations in 10 asci (cross 19, table 5) are 1 (4 : 0), 3 (3 : 1), 3 (2 : 2), and 3 (1 : 3). The genotype of the hybrid and therefore 1426A, with reference to the $MG_1\ MG_2$, and MG_3 loci, can be adduced from the following considerations. As in cross 18, the 4 : 0 and 3 : 1 ratios indicate that two polymeric genes or combinations of genes are heterozygous in this hybrid. However, the combination $MG_1\ MG_2\ MG_3/mg_1\ MG_2\ mg_3$ is precluded since a hybrid homozygous for MG_2 would not give 1 : 3 ratios. Thus the occurrence of 1 : 3 ratios indicates that MG_2 is not present in 1426A and, therefore, that a new gene MG_4 is responsible for the fermentation of alpha-methyl-glucoside in this haploid. The 1 : 3 ratios also eliminate the possibility that MG_4 is alone sufficient for fermentation. Does MG_4 act in concert with yet another new gene? Or, is MG_4 equivalent to MG_2 in requiring either MG_1 or MG_3 for alpha-methyl-glucoside fermentation? For example, if MG_4 and MG_2 are equivalent, all the above ratios can be accounted for by the genotype $MG_1\ mg_2\ MG_3\ MG_4/mg_1\ MG_2\ mg_3\ mg_4$

which would result in the following distribution of ascus types providing there was no case of linkage involved : 6 (4 : 0), 72 (3 : 1), 97 (2 : 2), 28 (1 : 3), and 1 (0 : 4). However, this interpretation was shown to be inadequate when the next cross of 1426A with 1018-2*b*, a complete recessive $mg_1\ mg_2\ mg_3\ mg_4$, gave 9 asci with these ratios : 3 (2 : 2), 1 (1 : 3), and 5 (0 : 4). If MG_4 is equivalent to MG_2, the situation would be analogous to that in crosses 11 and 12 and a preponderance of 2 : 2 segregations would be expected. The distribution of the segregation ratios requires either (1) that MG_1 or MG_3, but not both, is complementary to MG_4 if 1426A is $MG_1\ mg_2\ MG_3\ MG_4$, or (2) that neither MG_1 nor MG_3 are complementary to MG_4 and that a fifth gene must be postulated which in combination with MG_4 will permit fermentation. A further observation favoured the second possibility and provided evidence of the identity of the new gene. The asci from cross 20 (table 5) had 2 : 2 segregations for maltose fermentation (of the parents, 1426A was a fermenter whereas 1018-2*b* was not), and each alpha-methyl-glucoside fermenter obtained from this cross was also a maltose fermenter. The simplest interpretation is that the maltose gene segregating in cross 20, identified as MA_1, is involved in the fermentation of alpha-methyl-glucoside. This interpretation was supported by the results of intra-ascus zygote tests with the four haploids from a 0 : 4 ascus of cross 20 :

1028-9*a* (−) × 1028-9*b* (−)...(+) 1028-9*d* (−) × 1028-9*b* (−)...(−)
1028-9*a* (−) × 1028-9*c* (−)...(−) 1028-9*d* (−) × 1028-9*c* (−)...(+)

Under the hypothesis that 2 genes are needed to enable the haploids of 1028 to ferment alpha-methyl-glucoside, these results indicate that 9*a* and 9*c* have the same gene and that 9*b* and 9*d* have the other gene. The segregants 9*b* and 9*d* were maltose fermenters. Since the segregants 9*a* and 9*c* were maltose non-fermenters, the gene they carried was designated as MG_4.

To confirm the participation of the maltose gene MA_1 in the gene combination which enabled haploids of 1028 to ferment alpha-methyl-glucoside, a hybrid predicted to be homozygous for MG_4 and heterozygous for the complementary gene was obtained by crossing 1028-9*c* with 1028-6*c*, a fermenter of alpha-methyl-glucoside and maltose. It was expected that this hybrid would yield asci with 2 : 2 segregations for both alpha-methyl-glucoside and maltose fermentation, and if the above interpretation is correct, all the maltose fermenters would be alpha-methyl-glucoside fermenters. In the 11 tetrads that were obtained (cross 21, table 5), maltose segregated 2 : 2, but for alpha-methyl-glucoside there were 9 (2 : 2), and 2 (1 : 3) asci. Again every alpha-methyl-glucoside fermenter was also a maltose fermenter. In the 2 asci with 1 : 3 segregations, the haploids which were maltose fermenters but alpha-methyl-glucoside non-fermenters were found to be " petites " or cytochrome-deficient yeasts as described by Ephrussi, Hottinguer, and Chimenes (1949). Among the alpha-methyl-glucoside

fermenters from this hybrid as well as those from hybrid 1028, there were no petites although about 20 per cent. of the segregants from these hybrids were petites. In an experiment to investigate this relationship, a fermenter haploid, 1042-2*d*, was treated with acriflavine (Ephrussi, Hottinguer, and Tavlitski, 1949) to obtain a petite clone, 1042-2*d'*. This petite strain was then tested on alpha-methyl-glucoside and found to be a non-fermenter. When, however, this petite clone, 1042-2*d'*, and the spontaneous petite segregant, 1042-9*b*, from one of the 1 : 3 asci were checked by means of zygote tests with a normal strain of genotype $mg_1\ mg_2\ mg_3\ mg_4\ MA_1$, the presence of MG_4 was indicated since the cultures descended from the normal zygotes were capable of fermenting alpha-methyl-glucoside

1042-2*d'* (−) × 142-1*d* (−)...(+) 1042-9*b* (−) × 142-1*d* (1)...(+)

TABLE 5

Segregations for alpha-methyl-glucoside fermentation in asci from hybrids involving 1426A and its descendants

Cross	Parents and phenotypes	Hybrid and phenotype	Number of asci segregating as				
			4+ : 0−	3+ : 1−	2+ : 2−	1+ : 3−	0+ : 4−*
19	1426A (+) × 1018-4*a* (−).	1027 (+)	1	3	3	3	0
20	1426A (+) × 1018-2*b* (−).	1028 (+)	0	0	3	1	5
21	1028-6*b* (+) × 1028-9*c* (−)	1042 (+)	0	0	9	2	0
22	1042-2*d* (+) × 142-1*d* (−)	1044 (+)	0	0	4	1	0
23	1044-1*c* (+) × 136-4*c* (−)	1057 (+)	0	0	7	0	0

* Fermenters (+) : non-fermenters (−).

From these observations, it was concluded that strains of genotype $mg_1\ mg_2\ mg_3\ MG_4\ MA_1$ are unable to ferment alpha-methyl-glucoside if they are petites. It should be noted here that petite clones of the other fermenter genotypes are capable of fermenting alpha-methyl-glucoside although there is a delay of usually 3 or 4 days in the expression of the positive test.

To demonstrate the segregation of MG_4, crosses were made to give hybrids homozygous for MA_1 and heterozygous for MG_4. Of the 12 tetrads from crosses 22 and 23 (table 5) there were 11 in which there occurred the expected 2 : 2 segregation for the fermentation of alpha-methyl-glucoside. Again, in the 1 : 3 ascus from cross 22, one of the non-fermenters was a petite.

The presence of MG_3 in 1426A was established in the following manner. Zygote tests with the non-fermenters from a 2 : 2 ascus of 1028 indicated that one of the haploids carried a gene complementary to MG_2. This haploid, 1028-2*d*, was then crossed to a strain of genotype $mg_1\ MG_2\ mg_3\ mg_4$ and from this cross a fermenter segregant with the gene combination $MG_?\ MG_2$ was used in the test for allelism. The gene $MG_?$ was identified as MG_3 when the allelism tests with

1040-7*a*, genotype $mg_1\ MG_2\ MG_3\ mg_4$ gave only 4 : 0 asci (cross 25, table 6).

Evidence for an irregular segregation of MG_2

An unexpected 1 : 3 segregation was observed in one of the 14 asci from cross 26 (table 6), which had 1028-2*d* as the non-fermenter parent. The above tests have established the genotype of 1028-2*d* as $mg_1\ mg_2\ MG_3\ mg_4$. Since the genotype of the fermenter parent, 1037-2*c*, was $mg_1\ MG_2\ MG_3\ mg_4$ (see cross 17, table 4), only 2 : 2 segregations for the gene MG_2 were expected. The 1 : 3 ratio in the aberrant ascus could be due to a mutation affecting either MG_2 or

TABLE 6

Segregations for alpha-methyl-glucoside fermentation in asci from hybrids of the following genotypes: $mg_1\ mg_2\ MG_3\ mg_4/mg_1\ MG_2\ mg_3\ mg_4$ *in cross 24,* $mg_1\ MG_2\ MG_3\ mg_4/mg_1\ MG_2\ MG_3\ mg_4$ *in cross 25,* $mg_1\ MG_2\ MG_3\ mg_4/mg_1\ mg_2\ MG_3\ mg_4$ *in cross 26*

Cross	Parents and phenotypes	Hybrid and phenotype	Number of asci segregating as				
			4+ : 0−	3+ : 1−	2+ : 2−	1+ : 3−	0+ : 4−*
24	1028-2*d* (−) × 1093-2*c* (−)	1124 (+)	0	0	2	0	1
25	1124-3*d* (+) × 1040-7*a* (+)	1130 (+)	13	0	0	0	0
26	1037-2*c* (+) × 1028-2*d* (−)	1045 (+)	0	0	13	1	0

* Fermenters (+) : non-fermenters (−).

MG_3. To investigate these possibilities, zygote tests were made with the negative haploids from this ascus :

1045-9*a* (−) × 1040-6*a* (−)...(+) 1045-9*a* (−) × 1037-9*a* (−)...(−)
1045-9*b* (−) × 1040-6*c* (−)...(+) 1045-9*b* (−) × 1037-9*d* (−)...(−)
1045-9*c* (−) × 1040-6*a* (−)...(+) 1045-9*c* (−) × 1037-9*a* (−)...(−)

The zygote tests made with the $mg_1\ MG_2\ mg_3$ test strains, 1040-6*a* and 1040-6*c*, were positive for each of the three haploids. Those made with the $mg_1\ mg_2\ MG_3$ test strains, 1037-9*a* and 1037-9*d*, were negative. From these results, it can be concluded that the irregular segregation in this ascus was due to a mutation at the MG_2 locus.

Irregular segregations were also encountered for some of the other genes heterozygous in the foregoing hybrids. The genes for galactose (G_2) and melibiose fermentation, histidine, tryptophane (TR_1), and methionine independence each had a single 3 : 1 segregation. There were two asci with 1 : 3 segregations for uracil independence, and 3 asci with 4 : 0, 3 : 1, and 1 : 3 segregations for sucrose fermentation.

There was no significant evidence of linkage between any of the alpha-methyl-glucoside genes and any of the other genes segregating in these crosses : a/α mating type ; G_1 and G_2, *MEL*, SU_1, and MA_1, galactose, melibiose, sucrose, and maltose fermentation respectively ; tr_1 and tr_2, hi_1, ur_1, and me_1, tryptophane, histidine, uracil, and

methionine dependence respectively. The centromere linkage data were nor extensive enough to indicate significant centromere linkage for any of the alpha-methyl-glucoside genes.

4. DISCUSSION

It is possible to explain satisfactorily the segregations for alpha-methyl-glucoside fermentation encountered in some Saccharomyces hybrids on the basis of 5 interacting genes, designated MG_1, MG_2, MG_3, MG_4, and MA_1. The following combinations of dominant genes confer the capacity to ferment alpha-methyl-glucoside : $MG_1\,MG_2$, $MG_2\,MG_3$, and $MG_4\,MA_1$. The segregation ratios expected for a number of the possible heterozygous combinations have been given in the preceding sections. For a cross which gives a hybrid heterozygous for all 5 genes, $MG_1\,MG_2\,MG_3\,MG_4\,MA_1/mg_1\,mg_2\,mg_3\,mg_4\,ma_1$, the following distribution of segregation ratios is expected if the genes segregate independently and show no centromere linkage : 19 (4 : 0), 352 (3 : 1), 703 (2 : 2), 216 (1 : 3), and 6 (0 : 4).

The need for the presence of two interacting genes to enable a yeast strain to ferment a carbohydrate is not unique to the fermentation of alpha-methyl-glucoside. The fermentation of dextrin or starch by *Saccharomyces diastaticus* is also under the control of 2 genes, one controlling the splitting of the polysaccharide to maltose and the other controlling the fermentation of maltose (Gilliland, 1953). A more analogous situation is found for the fermentation of galactose where the presence of 2 dominant genes, G_1 and G_2, is essential for rapid fermentation (Leupold and Hottinguer, 1954 ; and Douglas and Condie, 1954). As a working hypothesis, the role of genes G_1 and G_2 was postulated by the latter investigators to be that G_1 controls the formation of an enzyme involved in the conversion of galactose to glucose-6-phosphate, and G_2 controls a mechanism for transporting galactose into the cell. As yet, the roles of the alpha-methyl-glucoside genes have not been investigated.

The presence of the gene combination $MG_4\,MA_1$ also enables a yeast strain to ferment sucrose although at a noticeably lower rate than when one of the specific sucrose genes is present : *i.e.* 48 to 72 hours are required for a full gas trap in a Durham fermentation tube with the $MG_4\,MA_1$ combination as compared to 16 to 24 hours with any one of the specific sucrose genes thus far isolated. It was postulated by Gilliland (1949) and demonstrated by Winge and Roberts (1952) that a maltose gene in their stocks, designated M_1, could enable a yeast strain to ferment sucrose slowly. Winge and Roberts (1955) established that this gene is allelic with the MA_1 of our stocks. However, in our stocks, strains with the gene MA_1 alone do not ferment sucrose at a detectable rate when tested in Durham tubes containing a medium consisting of 1 per cent. peptone, 1 per cent. yeast extract, and 2 per cent. sucrose.

The maltose gene and the 4 alpha-methyl-glucoside genes were isolated from stocks derived from 6 different parental strains. Two of the parental strains were obtained from Dr C. C. Lindegren and 4 were from stocks descended from strains originating in his laboratory. The source of these genes is of importance since Lindegren (1953) also has encountered irregular segregations for alpha-methyl-glucoside fermentation but has interpreted the deviations from a 2 : 2 ratio as cases of " gene conversion ". The irregularities in families with only a small proportion of anomalous asci, such as families VII and XI with 1 (4 : 0), 20 (2 : 2), and 2 (3 : 1), 30 (2 : 2) segregations respectively (table 1, p. 627, Lindegren, 1953), might reasonably be attributed to gene conversion. However, some of the cases that are cited may be explained on the basis of multiple genes. For example, the distribution of the segregation ratios in family XV (table 1, p. 627, Lindegren, 1953) with 1 (3 : 1), 20 (2 : 2), 18 (1 : 3), and 1 (0 : 4) asci would be expected, with the exception of the 3 : 1 ascus, from a hybrid heterozygous for the 3 genes MG_1, MG_2, and MG_3. In another case, the irregular segregations may be due to the occurrence of petite clones amongst segregants with the gene combination $MG_4\ MA_1$. In family XXVIII (table 1, p. 627, fig. 1, p. 628 Lindegren, 1953), 9 asci out of 13 gave 2 : 2 segregations for both maltose and alpha-methyl-glucoside and in each case, the alpha-methyl-glucoside fermenters were maltose fermenters. In the remaining asci, there were 1 (1 : 3) and 3 (4 : 0) segregations for alpha-methyl-glucoside fermentation. The fermenter parent in this cross, 1426, was shown above to be of genotype $mg_1\ mg_2\ MG_3\ MG_4\ MA_1$. If the non-fermenter parent, 12736, is of genotype $mg_1\ mg_2\ mg_3\ MG_4\ ma_1$, the observations of Lindegren can be accounted for by the occurrence of petite clones in the asci with the aberrant ratios. It has been noted in crosses with 1426 (crosses 19 and 20, table 5) that about 20 per cent. of the spore cultures are petite and frequently more than one petite spore culture arose from the same ascus.

The 9 asci where maltose and alpha-methyl-glucoside fermentation segregate together are cited by Lindegren as evidence supporting the location of *MA* and *MG* on the same chromosome. In the other families that he considers, there is no evidence of linkage for these genes from his summary of the data : 26 parental ditype, 33 non-parental ditype, and 99 tetratype asci. To account for this inconsistency of the data, a crossover suppressor is postulated for family XXVIII. It seems more probable, however, that different alpha-methyl-glucoside genes are being compared. These genes need not necessarily be MG_1, MG_2, MG_3, or MG_4 since it is highly probable that if in a sample of 6 strains there are found 5 different genes involved in alpha-methyl-glucoside fermentation, there exist other genes controlling this ability. Nevertheless, the irregular segregations in some of the families in the pedigree he presents may be explained on the basis of interacting genes, and this possibility must be kept in

mind when considering his data on alpha-methyl-glucoside fermentation as evidence pertaining to gene conversion.

5. SUMMARY

The distribution of the 2 : 2, 1 : 3, and 0 : 4 segregations for alpha-methyl-glucoside fermentation in the four-spored asci of Saccharomyces hybrids led to the postulation that 3 genes, MG_1, MG_2, and MG_3, are involved. A yeast with the dominant gene MG_2 and either dominant gene MG_1 or MG_3 is able to ferment alpha-methyl-glucoside. An additional gene combination enabling fermentation, $MG_4\,MA_1$, was discovered when a cross which yielded 4 : 0, 3 : 1, 2 : 2, and 1 : 3 ratios was investigated. Crosses were made which resulted in the segregation of a single gene at a time, and 2 : 2 ratios were obtained except for 4 anomalous 1 : 3 asci. Three of the anomalous asci involved the gene combination $MG_4\,MA_1$ and can be explained by the occurrence of petite spore clones from these asci since it was demonstrated that petite strains with this gene combination could not ferment alpha-methyl-glucoside. The irregular segregation in the fourth ascus is attributed to the mutation of the gene MG_2.

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INTRA-GENERATION INBREEDING EFFECTS IN A POULTRY FLOCK SELECTED FOR EGG PRODUCTION

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1. INTRODUCTION

It is inevitable that the levels of inbreeding within a selected closed flock should gradually rise above those of the initial stock. This effect is, of course, due to the finite size of the population, and to the fact that the genotypes selected represent only a part of the total gene pool of a generation. If, however, the flock is sufficiently large, and care is taken to restrict the mating together of closely related birds, the increased inbreeding may not be clearly detrimental. In poultry, egg production, fertility and hatchability of eggs, and vigour of offspring, may not necessarily show unequivocal decreases as the result of inbreeding. Indeed, these components of fitness may actually rise as a result of selection. Nevertheless, even if this is true it may still be possible, by appropriate statistical analysis, to demonstrate detrimental effects of inbreeding should they exist.

That inbreeding, generally speaking, has an adverse effect on many traits is well known. Jull (1933) and Stephenson *et al.* (1953) quote the works of a number of authors who attest to this in poultry. But almost all reports in the literature concern continuous close mating programmes, such as those of brother by half- or full-sister, and, consequently, a high rate of increase in the levels of inbreeding. The result, in these cases, is often that fitness is so greatly reduced that the existence of the line is in jeopardy. No studies of the effects of inbreeding when the rate of increase in the inbreeding levels is minimal for the size of the flock, when that size itself is relatively large, and when selection for some component of fitness has been effective, have apparently been attempted. But these are the effects which are likely to be operative in most cases of selection in closed flocks, and would therefore be pertinent to many aspects of selection problems and, indeed, to the future of closed flock breeding in general. An analysis of some of the effects has, therefore, been carried out, and is reported here.

2. MATERIALS AND METHODS

The University of California maintains a Single Comb White Leghorn flock which has been selected for egg production in each generation since its inception in 1932. Its history has been given in detail by Lerner (1950). Lerner and Hazel

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(1947) have tersely presented the essential features of the breeding plan, the following aspects of which are most relevant for the purposes of this study :

(1) Occasional outcrosses were made in the early years of the flock formation, the flock being closed after 1941 ;
(2) The size of the flock varied from 400 to 700 pullets each year from approximately 11 sires and 85 dams, with an exception of 260 pullets in 1944 ;
(3) Mating of closely related individuals was consciously avoided.

Lerner and Hazel note an average increase of 2·1 per cent. in the inbreeding coefficient per generation, or of about 1 per cent. per year when the average combined sire and dam inter-generation interval is taken into account.

The study reported here concerns data accumulated over a period of 18 years, from 1932 to 1949 and, in particular, after closure of the flock in 1941. In general, linear regression coefficients relating egg production (and its various components) and increases in inbreeding have been investigated. All inbreeding coefficients (Wright's F) were computed from the base year 1925. Since selection led to increasing egg production itself, even though this increase was accompanied over the generations by slowly rising coefficients of inbreeding, the analyses have been made on an intra-generation basis. Since in any one year there is no reason to expect a systematic relationship between inbreeding and selection pressure applied in the past, this method should largely eliminate bias which would otherwise come about from the parallel increase in F and in the characteristics under selection. It should be noted that, from studies of the relationship between family size and inbreeding (reported below), it was thought unnecessary, except in one case, to use any form of weighting in regression calculations.

3. RESULTS

1. *Number of birds.* Although members of each group of full sisters have an identical coefficient of inbreeding, the dam families in each generation show a range of values. At the time of a preliminary report on this study (Tebb, 1957) no evidence had been found for any relationship between pullet number and computed F. However, analyses on the data of earlier years show this lack of relationship applied only after closure of the flock.

Table 1, column (*a*), gives the linear regression coefficient of family size (pullet offspring only) against family F, for each year from 1932 to 1949. Twelve years show negative coefficients, but only one (1938, with a value of −0·195) reached the 5 per cent. level of significance.

Table 2 shows the regressions pooled within three arbitrary sub-periods and for the whole period. It should be noted that the occasionally significant F-test values for between years indicate that the regressions for different years within sub-periods are not necessarily the same. The regression for each group of years may therefore be viewed as an estimate of a weighted average of the regressions of the individual years, the weights being the within-years degrees of freedom for error, implied in the table. A significant F-test value for the pooled regression, based on the error mean square, indicates that this weighted average regression for the particular years is different from zero. An implicit assumption here is that the error variances are the same in all years.

TABLE 1

Yearly analyses of the flock between 1932-1949. The regression coefficients shown are those of: (a) *family size (pullets) on* F; (b) *mean hen-housed family total production (to 1st Oct.) on* F; (c) *mean hen-housed family part-production (to 1st Dec.) on* F; (d) *mean hen-housed family production during December on* F; (e) *family mean survivors' production (to 1st July) on* F; (f) *month of death on* F; *and* (g) *production to time of death on* F

Year	*F*		Number		Annual production	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)
	Range	Mean	Birds	Families								
1932	0-27	5·9	463	60	117·1	−0·110	0·661	−0·157	0·130			
1933	0-26	6·1	520	62	125·1	−0·148	−1·162	−0·605‡	−0·265‡			
1934	0-16	2·4	690	96	171·0	−0·291	−0·100	−0·332	−0·235			
1935	0-27	3·8	473	77	186·6	−0·019	−0·964	−0·310	−0·072			
1936	0-16	4·0	400	80	201·7	0·048	−1·626	−0·442	0·121			
1937	1-16	4·9	592	96	184·0	−0·012	0·276	0·130	−0·136			
1938	0-18	7·8	604	84	154·0	−0·195*	−0·563	0·597	−0·162			
1939	0-18	8·7	465	77	179·2	−0·017	−5·109†	−1·168†	−0·353†			
1940	0-33	9·6	428	79	195·9	−0·021	−0·727	0·416	0·128			
1941	0-22	10·6	638	108	213·5	−0·021	−1·211	−0·304	−0·148	−0·294	−0·073	−0·331
1942	0-17	9·1	424	74	208·7	0·151	2·564	1·176*	0·404*	1·332	0·017	1·598
1943	4-33	12·2	650	93	190·9	0·017	−1·437	0·214	−0·146	−0·035	0·040	0·680
1944	4-32	12·4	260	83	218·7	0·052	0·545	0·580	−0·072	0·332	−0·028	1·812
1945	2-27	13·4	445	92	214·8	0·030	−1·940	−0·474	−0·057	−0·583	0·021	−0·822
1946	10-29	14·0	479	91	203·7	−0·056	−3·558*	−0·662	−0·393*	−1·976*	−0·023	−0·094
1947	10-24	13·8	474	79	207·3	−0·189	−0·726	0·046	0·165	−0·500	−0·009	−0·103
1948	10-20	14·4	549	76	224·7	−0·195	−1·687	−0·467	−0·037	−1·898	−0·266	−3·696
1949	11-25	16·1	617	101	117·7	0·123	−0·588	0·241	−0·025	−1·356	−0·039	−0·116
1955	18-26	20·5	527	108	207·6	0·023	1·050					

* significant at $P<0.05$; † significant at $P<0.01$; ‡ significant at $P<0.001$.

From 1932-37, the mean annual coefficient of inbreeding ($\bar{F}$) rose from 2·40 to 6·12, and the pooled regression of family size (linear) of −0·089 is significant at the 5 per cent. level. From 1938-42 $\bar{F}$ rose to 10·59, and the pooled regression of −0·043 is also significant at the 5 per cent. level. From 1943-49 $\bar{F}$ rose to 16·11, and the pooled

TABLE 2

Analyses of the pooled yearly regressions, on computed F, *of* (a) *family size* (*pullets*), (b) *family mean total production* (*to 1st Oct.*), (c) *family mean part-production* (*to 1st Dec.*), (d) *family mean production during December. The three sub-periods and the total period are analysed separately*

Period		Pooled within-year regression		Yearly regression differences		Error	
		Value	M.S.	d.f.	M.S.	d.f.	M.S.
1932-37	(*a*)	−0·089	78 *	5	18	459	12
	(*b*)	−0·618	3748	5	1166	459	1969
	(*c*)	−0·335	1105 †	5	108	459	158
	(*d*)	−0·103	104 *	5	46	459	25
1938-42	(*a*)	−0·043	51 *	4	10	412	10
	(*b*)	−1·157	11,202 *	4	6837 *	412	2848
	(*c*)	0·033	9	4	906 †	412	219
	(*d*)	−0·080	54	4	85 *	412	27
1943-49	(*a*)	0·005	0	6	10	601	78
	(*b*)	−1·330	13,195 *	6	1847	601	2657
	(*c*)	0·037	10	6	171	601	167
	(*d*)	−0·098	72	6	24	601	23
1932-49	(*a*)	−0·047	56 *	17	16	1472	10
	(*b*)	−1·001	25,689	17	2748	1472	2496
	(*c*)	−0·107	292	17	354 *	1472	179
	(*d*)	−0·094	228 †	17	42 *	1472	25

* significant at $P<0·05$
† significant at $P<0·01$

regression is positive (0·005) but not significant. When all years are combined, the regression coefficient of −0·047 is also significant at the 5 per cent. level. In no period is the mean square of the differences between annual regressions significant.

Table 1, column (*a*), includes the coefficient for 1955, and this is slightly positive but statistically insignificant. Thus it seems that whatever the dependence of family size on inbreeding has been, it apparently ceased after closure of the flock. In view of the objections to weighting, of the relatively small ranges in family size, and of the independence between family size and F, in the later stages, unweighted regressions have been used chiefly but not entirely.

2. *Annual production index.* Table 1 gives essential data pertaining to the flock in each year. Differences in some of the earlier years from similar data in Lerner and Dempster (1951) are due to differential treatment of floor-layers in the two cases.

Table 1, column (*b*), shows the linear regression coefficients of mean family production on *F*. The production figures used were those from beginning of lay to October in the first laying year, and the family means were based on the numbers of pullets initially banded, *i.e.* the production index (or hen-housed average) was used. There are 4 positive, and 14 negative coefficients. In only two years are these coefficients statistically significant: in 1939 (−5·109) at the 1 per cent. level, and in 1946 (−3·558) at the 5 per cent. level.

Table 2, line (*b*), gives the pooled analyses for the three sub-periods and for all years together. From 1932-37, neither the joint regression (−0·618) nor the differences between annual regressions are significant. Both the joint regression (−1·157) and the annual differences are significant, at the 5 per cent. level, from 1938-42. The joint regression (−1·330) is alone significant, at the 5 per cent. level, from 1943-49. Finally, the overall regression for all years is −1·001, and significant at the 1 per cent. level, annual differences being statistically insignificant.

Since sire differences are almost certain to have existed, an analysis of intra-sire relationships between *F* and production was performed. Briefly, all pullets of a sire were divided into two groups, those of higher and those of lower *F*, and the mean egg production of each group found. In each year, therefore, some sires had offspring with a lower *F* value having the higher production, while lower *F*-value pullets from other sires had poorer production than their half-sisters. If inbreeding had no effect on egg production, there should be no significant deviation from a 1 : 1 ratio between these groups of sires. Table 3 shows the results, the numbers under " lower " and " higher " indicating the numbers of sires in each year for which pullets with higher or pullets with lower *F* values had the superior production. In 13 years more sire families had superior production amongst the pullets of lower *F*. In three years the reverse was true. In two years (1940 and 1948) equal numbers were found. In general, this table shows results comparable to those which might have been anticipated from the actual regression coefficients of table 1, column (*b*). Over all years, pullets from 121 sires had higher production than their half-sisters with a higher *F* value; the reverse was true for pullets from 77 sires. The test of significance at the bottom of table 3 shows that the χ^2 value for the deviation from a 1 : 1 ratio in all years was 9·778, significant at the 1 per cent. level. On the other hand, the χ^2 value for heterogeneity between years, 19·594 for 17 degrees of freedom, is not statistically significant.

In table 4 regression coefficients of mean production weighted by pullet number per family, together with the pooled weighted regression, are shown for 9 of the years. Since, where an association exists, family size and *F* appear to be negatively correlated—higher inbreeding being associated with smaller family size—it might be anticipated that a regression weighted by pullet number would show coefficients which were displaced in the negative direction. This is,

in general, to be seen in table 4. The pooled weighted regression for all 9 years has a coefficient of −1·174, compared with the pooled unweighted coefficient of −1·001 for all 18 years, and reaches significance at the 1 per cent. level. That annual production is decreased by inbreeding, therefore, appears a valid conclusion.

TABLE 3

Analysis of dam families within sires, as described in the text. χ^2 values are for deviation from a 1 : 1 ratio

Year	Lower	Higher	Sires	χ^2
1932	6	5	11	0·091
1933	8	1	9	5·444
1934	7	2	9	2·778
1935	5	3	8	0·500
1936	8	2	10	3·600
1937	5	7	12	0·333
1938	9	3	12	3·000
1939	10	2	12	5·333
1940	7	7	14	0
1941	9	3	12	3·000
1942	3	6	9	1·000
1943	8	5	13	0·692
1944	3	7	10	1·600
1945	6	5	11	0·091
1946	6	4	10	0·400
1947	7	4	11	0·818
1948	6	6	12	0
1949	8	5	13	0·692
All years	121	77	198	29·372

	d.f.	χ^2
Deviation from 1 : 1 ratio . .	1	9·778 $p<0·01$
Heterogeneity between years .	17	19·594 $p\simeq0·3$
Total . . .	18	29·372

Table 2, line (*b*), shows that the coefficients of the pooled unweighted regressions of mean family production on *F* have increasing negative values for the three consecutive sub-periods : −0·618 from 1932-37, −1·157 from 1938-42, and −1·330 from 1943-49. These results suggest that the relationship between production and inbreeding may be better described in a polynomial, rather than a linear, regression. No satisfactory scale transformation could be found to apply with consistency equally well to all years. It seemed preferable, also, to investigate any possible nonlinearity in the relationship of production and *F* by studying whole generations. Table 5 shows the sums of squares of the linear and quadratic components of mean egg production per family on *F*, for the years 1942-48, and the " error " mean square for each component. The method used was that described by Mather

(1949). Of the 7 years investigated, 4 show that the quadratic component removed a greater sum of squares than did the corresponding

TABLE 4

The result of weighting the mean hen-housed family production to 1st October by the number of pullets in the family, and calculating the regression of this weighted production mean on the family F, *is shown here for each of several years between 1932 and 1949, together with the pooled regression of all the chosen years combined*

Year	Regression		Error	
	Value	M.S.	d.f.	M.S.
1934	−0·525	1674	94	7911
1936	−2·667	42,810 *	78	8008
1938	−0·764	5357	82	14,871
1940	−1·100	8850	77	15,690
1943	0·108	96	91	6784
1944	1·707	13,453	81	8358
1946	−5·036	108,539 †	89	9595
1947	−2·236	19,490	77	10,795
1948	−2·225	11,940	74	10,059
			743	10,121
All years : −1·174 M.S. for joint regression : 71,794 † M.S. for differences between regressions : 17,552				

* significant at $P<0·05$
† significant at $P<0·01$

TABLE 5

The effect of fitting quadratic regression of mean hen-housed family production (to 1st Oct.) on F, *for the years 1942-1948. Analysis of both linear and quadratic components are shown, and the numbers of the degrees of freedom in each case are shown in parentheses*

Year	Linear component		Quadratic component	
	Regression S.S.	Error M.S.	Regression S.S.	Error M.S.
1942	4707	2248 (72)	4733	2213 (71)
1943	2842	1993 (91)	5830	1950 (90)
1944	384	3904 (81)	36	3953 (80)
1945	6581	2130 (90)	7846	2065 (89)
1946	12,783 *	3028 (89)	20,332 †	2831 (88)
1947	313	2609 (77)	0	2678 (76)
1948	995	2166 (74)	929	2183 (73)

* significant at $P<0·05$
† significant at $P<0·01$

linear component : but only in 1946 did either component reach statistical significance : the linear at the 5 per cent. and the quadratic at the 1 per cent. levels.

3. *Other measures of production.* Table 1, column (*c*), shows the linear regression coefficients of mean family part-production (from first egg to the 1st December) on *F* from 1932-49. The means were again based on the initial family size. Only 10 years have negative coefficients, five of them being in the period 1932-36. The coefficients are, however, statistically significant in only 3 years: −0·605 in 1933 ($P<0·001$); −1·168 in 1939 ($P<0·01$); and 1·176 in 1942 ($P<0·05$). Table 2, line (*c*), shows that, from 1932-37, the pooled regression of −0·335 is statistically significant at the 1 per cent. level, differences between yearly regressions not reaching significance; that, from 1938-42, the differences between years but not the pooled regression of 0·033, are significant at the 1 per cent. level; and that, from 1943-49, neither the pooled regression of 0·037 nor the yearly differences reach statistical significance. The pooled regression for all years is −0·107 and not significant, but the yearly differences are significant ($P<0·05$).

Analysis of December production itself might yield information about the association of rate of production and pausing, with inbreeding. The coefficients of regression using this measure of production are shown in table 1, column (*d*). Only 3 of the 13 negative coefficients reach statistical significance: −0·265 in 1933 ($P<0·001$), −0·353 in 1939 ($P<0·01$) and −0·393 in 1946 ($P<0·05$). One of the 5 positive coefficients, 0·404 in 1942, is significant at the 5 per cent. level. Table 2, line (*d*), shows that, from 1932-37, the pooled regression of −0·103 is significant at the 5 per cent. level, yearly differences not reaching significance; that, from 1938-42, the pooled regression of −0·080 is not significant, but the yearly differences reach the 5 per cent. level; and that, from 1943-49, the regression (−0·098) and the yearly differences are, alike, statistically insignificant. The pooled regression coefficient for all years is −0·094 and significant ($P<0·01$), the differences between years also reaching significance ($P<0·05$).

Table 1, column (*e*), shows the yearly coefficients of the linear regression of survivor production on *F*. Survivors were defined as those birds alive on 1st July of the initial laying year, and production to that date was used. In some years after 1941 partial culling took place between July and October. These were, in almost every case, sick birds showing abnormalities at autopsy, and might have been expected to die before October; nevertheless, the earlier month of July was chosen, as a criterion for determining a survivor, in order to eliminate bias. In occasional years prior to 1941 a few birds were culled earlier than July, in order to make room for new stock. Prior to 1941, therefore, no survivor regressions were calculated. Of the 9 years analysed, 7 have negative regressions. The coefficient of −1·976 in 1946, alone, reaches significance, at the 5 per cent. level. Table 6, line (*a*), shows that neither the pooled regression of these years (−0·412), nor the yearly differences, are statistically significant.

4. *Production of dead birds.* An attempt, shown in table 7, was made to determine whether the birds which died had, on the average,

TABLE 6

The pooled regressions for the years 1941-1949 of (a) *family mean survivors' production to 1st July on* F, (b) *month of death on* F, *and* (c) *production to time of death on* F

	Pooled regression		Yearly differences		Error	
	Value	M.S.	d.f.	M.S.	d.f.	M.S.
(*a*)	−0·412	1817	8	1905	761	994
(*b*)	−0·016	4	8	10	836	12
(*c*)	0·025	11	8	1436	836	4094

higher F values than the mean for the whole of the flock in their generation. For each year from 1941 the mean F of all birds dying

TABLE 7

The mean F *of all birds which died before* (a) *1st January, and* (b) *1st July, compared with the mean* F *of the whole flock when each family* F *is weighted by its pullet number*

Year	(*a*) 1st January		(*b*) 1st July		(*c*) Whole flock	
	No.	F	No.	F	No.	F
1941	37	12·303	130	11·315	638	10·626
1942	23	9·084	58	9·076	424	9·101
1943	31	10·338	104	11·779	650	11·737
1944	9	12·338	49	11·716	260	12·367
1945	30	13·001	98	13·901	445	13·398
1946	32	15·106	61	14·464	479	14·008
1947	43	14·013	107	13·891	474	13·838
1948	30	14·426	86	14·207	549	14·433
1949	63	15·819	161	16·158	617	15·860
All years	298	13·426	854	13·291	4536	12·880

before (*a*) 1st January, and (*b*) 1st July, are compared with the flock mean, the latter obtained by weighting each family F by the initial family size. If inbreeding contributes to increased mortality, the mean F of (*a*) should be higher than for (*b*), and both higher than the flock mean. Only three years (1941, 1946 and 1947), however, show this trend, although 6 years show an $\bar{F}$ of birds dying before July which is greater than that for the whole flock. Combining all dead birds in all years, the $\bar{F}$ of birds dying before January is 13·426, before July is 13·291, and for the flock in all years, 12·880, suggesting that inbreeding is accompanied by higher death rate and also by earlier age at death.

To compute regressions of month of death of dead birds on F, each month was numbered in sequence. Table 1, column (f), shows the linear regression coefficient of month of death of dead birds on the F of the family of which each bird was a sister. Six of the 9 years from 1941-49 have negative regression values, none reaching statistical significance. Table 6, line (b), shows the pooled analysis for all 9 years. Neither the pooled coefficient (−0·016) nor the differences between years, are statistically significant.

TABLE 8

Within-year analyses of proportions of deaths between unit differences of F (*see text*). *The table shows, for each year from 1941-1949 and for 1955, the regression of proportion of dead birds in families on the family* F *unit group, and the significance of the heterogeneity between proportions*

Year	Regression		Heterogeneity	
	Value	M.S.	d.f.	S.S.
1941	0·0069 *	0·895	19	9·634 ‡
1942	−0·0067	0·165	12	2·698
1943	−0·0057	0·263	15	4·546
1944	−0·0066	0·205	15	2·392
1945	0·0045	0·183	17	4·330
1946	0·0078	0·308	14	2·059
1947	0·0078	0·242	12	1·989
1948	0·0023	0·013	10	2·791
1949	0·0039	0·106	14	6·991 †
1955	−0·0027	0·028	7	5·019 ‡

* significance at $P<0·05$
† significance at $P<0·01$
‡ significance at $P<0·001$

Table 1, column (g), gives the yearly coefficient of the regressions of production to time of death of dead birds, on F. Six years show negative coefficients, none reaching statistical significance. Table 6, line (c), shows the pooled analysis for the 9 years. Neither the pooled regression coefficient (0·025), nor the yearly differences, reach significant levels.

Lastly, an attempt to determine whether families of higher inbreeding contained, on the average, a greater number of birds dying before July is summarised in table 8. The method outlined by Armitage (1955) was used, the essential question asked being whether or not the proportion of birds dying within each year showed a significant trend with the increase of the family inbreeding value. In each year families were gr uped in unit percentage differences of F : for example, all families in 1941 the F of which was between 7·000 and 7·999 are pooled, and give a total of 24 deaths from an initial pullet number of 78, or a mortality proportion of 0·308 and an F of 7·500. A linear regression of this proportion on F can then be found, together with

the significance of the heterogeneity present. As shown, 6 of the 9 years have positive coefficients (suggesting that the proportion of deaths increases with an increasing F), only that for 1941 (0·0069) reaching the 5 per cent. level of statistical significance. Furthermore, heterogeneity is highly significant in 1941 ($P<0·001$) and in 1949 ($P<0·01$). The value for 1955 was also computed in order to see if the positive regressions of the years 1945-49 are maintained : but the coefficient is negative and insignificant, heterogeneity in that year being highly significant ($P<0·001$).

4. DISCUSSION AND CONCLUSIONS

The factors determining total number of offspring reaching reproductive age are known to be numerous and include the number, fertility and hatchability of eggs, and rearing viability. At least some of these components of fitness may be adversely affected by inbreeding. Düzgüneş (1950) found that, in general, in the intensely inbred lines which he analysed, inbreeding tended to reduce the numbers of offspring which survived to breeding age, largely as a result of decreased hatchability, although other characters were also affected to some extent. If, however, as in this report, female offspring alone are considered, additional variance will be introduced by chance deviations in the sex ratio. Since, therefore, the pooled regression of family size (pullets only) on F in all years is statistically significant (table 2), inbreeding effects appear to have been pronounced enough to be demonstrable in spite of this chance variation in sex. When the period is broken down into 3 sub-periods only the first 2 of these show pooled significance. It seems reasonable to explain the absence of significant trends during the last sub-period as being due to the more restricted range of F values in these years (table 1). In other words, it is probable that in earlier years the range of inbreeding was great enough for inbreeding effect on pullet number to be revealed in spite of deviations in the sex ratio ; in later years the range became too narrow, and chance was the chief determinant.

The analyses reported here clearly show that inbreeding has had a detrimental effect on production within generations. Lerner and Hazel (1947) have previously calculated the regressions of mean family production on the computed F for 4 years of this flock. In doing so, they excluded all families of less than 5 pullets, and obtained coefficients of −0·54, −0·46, −5·81 and 0·84 for 1935, 1937, 1939 and 1941, respectively, of which only the 1939 figure was statistically significant. The coefficients of table 1 for these years are, in order, −0·964, 0·276, −5·109 and −1·211, the 1939 figure again being the only one of significance. The average for the four years of Lerner and Hazel was −1·20, and this was statistically insignificant. The authors, therefore, concluded that there appeared to be insufficient evidence to suggest that inbreeding was important in limiting production. The more extensive analyses of this report justify the conclusion

that a linear regression of about one egg for every per cent. increase in computed F can be detected in the means of family production. This rate, incidentally, is very similar to those reported for mixed inbred lines by others. Shoffner (1948) for example, found the within-sire regression of full-sister mean family production on the mean inbreeding value to be $-0{\cdot}926$.

Mean egg production of dam families, taken to October of the first laying year and based on the initial pullet numbers per family is, of course, a composite figure. Several or all of the following may occur :

1. rate of production may be higher in less inbred birds than it is in birds of greater inbreeding ;
2. birds of lower levels of inbreeding may reach sexual maturity earlier than do birds of higher inbreeding levels ;
3. the proportion of deaths may be higher amongst birds of greater inbreeding levels than amongst those of lower levels ;
4. deaths amongst birds of higher inbreeding levels may be earlier than those amongst lower levels, so that the former group lay fewer eggs than the latter ;
5. the rate of production of dead birds may be higher amongst birds of lower inbreeding levels than amongst those of higher ;
6. there may be a complex interaction of all or some of these factors.

The bottom line of table 2 shows an overall significant regression of mean family December production on F and, at the same time, significant differences between years. December egg production includes, of course, not only rate of lay when in lay but also the effect of possible winter pausing. That yearly differences are significant can possibly be a reflection of climatic conditions : when production drops due to unfavourable conditions, more highly inbred birds may be affected the most ; when the season is less severe laying may be less subject to effect by inbreeding. A longer period of time, such as the whole year, may contain successive sub-periods of unfavourable, favourable and indifferent climatic conditions, and analyses of this longer period may be obscured by the changes in conditions it embraces, while yearly differences in these analyses may be further obscured. It is possible, therefore, that within a smaller interval of time, such as December, climatic conditions may determine much of the year-to-year differences encountered. In other words, rate of lay when birds are laying may be but little affected by inbreeding, whilst the tendency to react to bad seasonal effects by pausing may be largely determined by inbreeding level, the more highly inbred birds pausing first. Further work is, however, necessary to verify this. At the same time, the December regression of all years combined is itself highly significant in table 2, and gives grounds for thinking that inbreeding has also an influence directly on rate of production during this month.

Inbreeding, in view of the insignificant pooled coefficients of regression to December in table 2, would appear to have little effect on sexual maturity. There was, however, a highly significant regression for the pooled analysis of the first sub-period, 1932-37. An explanation could be that, in earlier years the wider range of F values revealed inbreeding effects during these years ; though there are many further complications in this character (*e.g.* the effect of date of hatch).

The suggestion in table 7, that more and earlier deaths occur amongst birds of higher inbreeding levels than amongst those of lower, is not statistically established by the analyses reported in tables 6 and 8, nor is the relationship between the production of either surviving birds or birds dying in the course of the year and the F values statistically significant.

Hence, although there is ample evidence that inbreeding affects egg production when the latter is measured as a hen-housed average, the precise manner in which it does so is not clearly apparent. It is interesting that, in all calculations, there are indications of the detrimental effect of inbreeding, even though these indications rarely reach statistical significance. The majority of years show negative regression coefficients for nearly all traits examined, with the exception of table 8, where in this case the detrimental effects of inbreeding would be revealed by positive signs for the coefficients of regression. There is, therefore, an underlying consistency in the results, and much yearly difference. The underlying consistency may be spurious, but both this and the yearly heterogeneity point to the same tentative conclusion. This conclusion, which requires further work before validation, is that inbreeding has an overall debilitating effect on nearly every factor contributing to production, but chiefly that it carries a penalty in times of stress.

A consequence resulting from the adverse effects of inbreeding on production is that the birds selected from a given generation are likely to be in the lower range of F values for that generation, and the unselected birds in the higher. To test this, a short survey was made of the years 1943, 1944, 1945 and 1946, the results being as follows :

Hatch	Unselected pullets		Selected pullets		Mating
	No.	$\bar{F}$	No.	$\bar{F}$	
1943	583	12·211	67	12·495	1945
1944	202	12·375	58 *	12·338	1946
1945	377	13·498	68	12·845	1947
1946	404	14·154	77	13·199	1948

* This figure differs from that in 1944 reported by Lerner and Dempster (1951) in their table 4, because of 16 birds included in their report for which the inbreeding coefficients were not available.

In 3 of the 4 years the $\bar{F}$ of selected pullets is slightly less than that for unselected sisters.

There are two possibilities which should also not be overlooked in examining the results reported. The first of these is that linear regression may not be adequate to describe the relationship of inbreeding and factors contributing to production. The results of table 5 are far from conclusive. Yet Stephenson *et al.* (1953) reached the decision that in their material the regression did not become linear until the *F* value had reached 25 per cent., although they were concerned specifically with egg production rate and their lines were more rapidly inbred. Secondly, the differences between the actual increases in homozygosity and the computed *F* value may not be proportionally the same throughout the period studied.

5. SUMMARY

1. A S.C.W. Leghorn flock of the University of California has been subject to continuous selection for egg production since 1932. Inbreeding increases, however, have been kept at a minimum consistent with the size of the flock and the selection pressure exercised.

2. Intra-generation regressions of mean family production to the start of October in the first laying year on the computed *F* values, for each of the years 1932-49, have mostly negative coefficients. Coefficients of only 2 of the 18 years reached statistical significance but the pooled regression for the total period showed a highly significant negative coefficient of about one egg for every per cent. increase in the computed *F*.

3. Family size, when pullets alone are considered, appeared to be correlated with the family *F* value prior to 1942, but uncorrelated in later years. It is suggested that, in later years, the narrower range of *F* values, and chance deviations in the sex ratio, contributed to remove any real effect of inbreeding on family size. There was a statistically significant negative regression coefficient of −0·047 pullets for every increase in per cent. *F*, when all years were combined.

4. Within-sire analyses corroborated the presence of an adverse effect of inbreeding on production.

5. Quadratic regression does not, in the data analysed, appear to be a closer description of the relationship between production and inbreeding, possibly due to the large amount of yearly heterogeneity present.

6. No significant regressions of survivors' production, month of death and production to death of dead birds, or per cent. viability on *F*, were observed. It can be shown, however, that over all years, the mean *F* of birds dying was slightly greater than that of birds surviving, and that the mean *F* of birds dying earlier was slightly greater than that of birds dying later.

7. It is concluded that, although even slow inbreeding detrimentally affects the hen-housed production within any year, the precise manner in which it does so is not clearly apparent. An underlying consistency

in the material investigated is accompanied by yearly heterogeneity. Inbreeding probably affects adversely nearly every factor contributing to total production. It is suggested that its severest consequences are found when environmental and local conditions create situations involving stress.

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THE PRODUCTION AND PHENOTYPES OF WHEAT-RYE CHROMOSOME ADDITION LINES

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1. INTRODUCTION

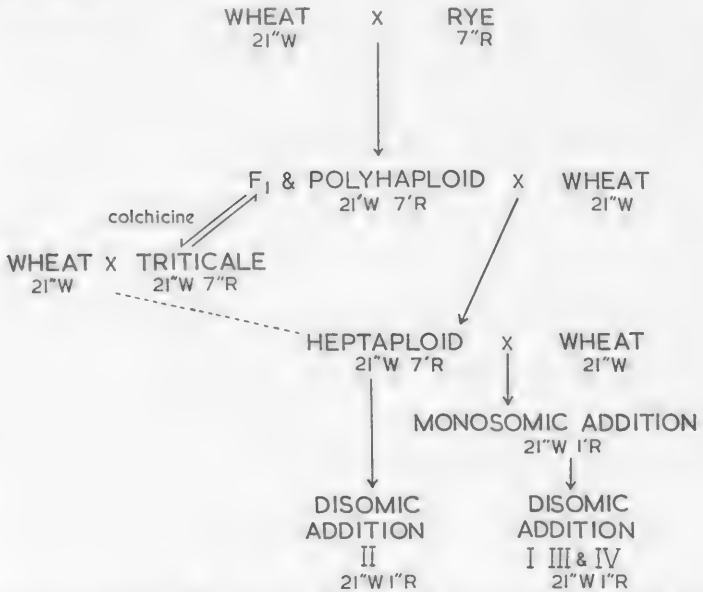
It is impossible to transfer characteristics of rye, *Secale cereale* a diploid, to wheat, *Triticum vulgare* a hexaploid, by normal breeding methods since there is very rarely, if ever, any meiotic pairing between the chromosomes of the two species in the intergeneric hybrid. This has been particularly tantalising to plant breeders because there are characters of rye which it would be economically valuable to incorporate in wheat. Consequently the amphidiploids derived from wheat-rye hybrids by chromosome doubling have attracted considerable attention (Rimpau, 1891; Lindschau and Oehler, 1935; Müntzing, 1939 and 1948; Riley and Chapman, 1957), since in these plants the complete gene-complements of wheat and of rye are associated in fertile combinations. However, because of their low fertility and the genetic antagonism of the parental genotypes, wheat-rye amphidiploids have been of no value in agriculture. Analogous amphidiploids between wheat and related primitive species in the genus *Ægilops* are similarly of no direct value (Bell, Lupton and Riley, 1955) because disadvantageous features of the primitive species are included together with the desired characters.

It was proposed by O'Mara (1940) that the undesirable features of wheat-rye and other amphidiploids might be overcome by incorporating with the wheat genotype not the whole genome of another species but the smallest part of the genome which was necessary in order to introduce the desired character. There would then be the minimum alteration of the basic phenotype of the wheat. For a character, controlled by a single gene, to be introduced from a species such as rye, the chromosomes of which are not homologous with those of wheat, the problem resolves itself into the transfer to wheat of a single pair of alien chromosomes. It was proposed that the alien pair should be added to the full complement of wheat chromosomes to produce what R. E. Clausen (Gerstel, 1945) has referred to, in *Nicotiana*, as an "alien addition" line. Because of their balanced chromosome constitutions such lines might be expected to be true-breeding and of normal fertility.

O'Mara (1940 and 1951) successfully demonstrated that by a sequence of manipulations in which the wheat-rye amphidiploid was crossed with wheat and the derivatives allowed to self for one or more generations it was possible to obtain plants which had the full

complement of wheat chromosomes plus rye chromosome I, monosomic or disomic. Subsequently Chapman and Riley (1955) have obtained the disomic addition of rye chromosome II to wheat, using essentially the same technique as O'Mara.

Techniques differing from O'Mara's have been used by Hyde (1953) to add five of the chromosomes of *Haynaldia villosa* to wheat, by Sears (1956) to add a chromosome of *Ægilops umbellulata* to wheat, and by Gerstel (1945) to make chromosome transfers between species of *Nicotiana*.



TEXT-FIG. 1.—The breeding technique illustrated was used to extract plants with pairs of rye chromosomes separately added to the full complement of wheat chromosomes. The present addition lines, shown by roman numerals at the disomic addition level, were obtained by the sequence marked with continuous lines between generations. Subsequent work was based on crossing the wheat-rye amphidiploid with wheat to obtain heptaploids, as indicated by the dotted line. (W = wheat and R = rye chromosomes, ″= bivalents and ′= univalents at meiosis.)

The transfer of individual pairs of chromosomes from one species to another is thus possible, at least in some groups, and it is apparent that the technique may not only be of value in plant breeding but that it also permits the creation of novel situations of cytogenetic interest. For in material of this type each chromosome pair of the donor species can be isolated from the rest of the complement and examined separately against the genotypic background of the recipient species. It may thus be possible to determine the linkage groups of genes the expressions of which are epistatic to those of the recipient species, or which interact with genes of the recipient species. Since

the added chromosome will sometimes be morphologically recognisable, particular genes may be related to particular chromosomes, a task which is difficult using euploids. Also, as with the study of chromosome races within species, such as trisomics (Blakeslee and Avery, 1934 ; Blakeslee, 1934) and monosomics (Sears, 1954), genes may be located which are inaccessible to formal genetic study since only one allele is known. Further, the behaviour of chromosome pairs transferred to a foreign genotypic background is of cytogenetic and evolutionary interest.

It was for both practical and theoretical reasons, therefore, that the present studies on the transfer of individual chromosomes from rye to wheat were undertaken. The present paper is concerned with the production and phenotypes of lines in which single rye chromosomes, or pairs of chromosomes, have been separately added to the full complement of wheat chromosomes.

2. MATERIALS AND METHODS

The two species concerned in the present chromosome manipulations are :—

Secale cereale L. strain King II ($2n = 14$).

Triticum vulgare Vill. variety Holdfast (= *Triticum æstivum* L. emend, Fiori and Paoletti) ($2n = 42$).

Meiotic examinations were made on permanent Feulgen squashes of pollen mother cells, in which 1 per cent. orcein in 45 per cent. propionic acid was used for the final squash instead of the usual 45 per cent. acetic acid. Root-tips for chromosome counting were similarly stained after being pre-treated in α-bromonaphthalene.

3. THE PRODUCTION OF ADDITION LINES

The method used to obtain addition lines deviated somewhat from that proposed by O'Mara (1940) since in the present work a 28-chromosome polyhaploid, which arose in the amphidiploid derived from Holdfast and King II, was crossed with Holdfast (fig. 1). This polyhaploid plant was like the species-hybrid in phenotype and in the frequency of bivalents at meiosis, and very probably combined the haploid complements of wheat and rye.

One plant was derived from the cross between the polyhaploid and Holdfast wheat, and this was a heptaploid with 49 chromosomes. Since 21 of the chromosomes of the heptaploid were received from the wheat parent an unreduced gamete with the full set of 28 chromosomes must have been received from the polyhaploid. At meiosis the chromosomes from the wheat parent paired with 21 of the chromosomes from the polyhaploid, and seven of the chromosomes from the polyhaploid remained as univalents. This plant then had the full complement of 42 wheat chromosomes and the haploid set of seven rye chromosomes.

This diagnosis of the constitution of the heptaploid was confirmed by the behaviour of two 42-chromosome plants obtained by crossing it with Holdfast, for at meiosis these derivatives had 21 bivalents, and were phenotypically similar to Holdfast. These plants had

returned to the chromosome constitution of wheat, and since the 21 chromosomes they had received from the heptaploid must have been involved in bivalents in that parent the bivalents are shown to have been composed of wheat chromosomes, and the univalents of rye chromosomes.

The seven rye univalents in the 49-chromosome plant were included in the gametes, or excluded, at random, so that when it was crossed again to wheat, or allowed to self, 25 aneuploids with chromosome numbers ranging from 42 to 52 were obtained. The two 42-chromosome derivatives have been discussed above and only the 43- and 44-chromosome forms from which addition lines were developed have here been considered further.

Seven of the plants derived from the heptaploid had 43 chromosomes which formed 21 bivalents and one univalent at meiosis. There were three readily distinguishable phenotypic categories, and the lines developed from these plants have confirmed the distinctiveness of the classes. These 43-chromosome plants were monosomic for the addition of one or other of three rye chromosomes to the full complement of wheat. Subsequently the 43-chromosome plants were allowed to self and amongst their progenies were small proportions of individuals with 44 chromosomes which made 22 bivalents at meiosis. In each case, therefore, the added rye chromosome had been obtained as a disomic addition to the full wheat complement, and this condition was to varying extents stable from generation to generation. Three of the addition lines, described below, were derived from the initial monosomic addition plants.

The other addition line extracted from this material was obtained directly as a 44-chromosome disomic addition of rye chromosome II, as a result of selfing the heptaploid plant.

Four distinct rye chromosome addition lines, of the seven possible, were thus established from the first or second generation derivatives of the original heptaploid plant. Disomic additions of each chromosome were crossed together to test their distinctiveness and the formation of 21 bivalents and two univalents in the interline hybrids confirmed that a different rye chromosome had been added in each case. Disomic additions of each line were crossed with Holdfast wheat and in the 43-chromosome F_1's 21 normal bivalents and one univalent were formed. This confirms that the wheat chromosome complement remained intact and structurally undisturbed in the addition lines, and that an alien pair of chromosomes had indeed been added.

4. THE PHENOTYPES OF THE ADDITION LINES

In the following descriptions of the phenotypes of the addition lines the only characters that have been considered are those which are altered in the addition lines compared with Holdfast, the wheat parent (table 1). Attention has therefore been concentrated on the

TABLE 1

The phenotypic modifications of Holdfast wheat due to the separate disomic addition of particular rye chromosomes

Chromosome	Vegetative characters		Ear characters		Grain
	Juvenile plant	Mature plant	Ear conformation	Spikelets	
I	Leaves pale green, plants faster growing	Straw stiffer	Longer ($1\frac{1}{4}\times$ Holdfast), very lax particularly at base, hairy neck	...	Slightly larger
II	Faster growing, erect habit, leaves dark green, short and broad and held stiffly upright	Slightly taller, straw robust	More tapering, slightly narrower	Apical points of glumes blunter, lemma points longer	Smaller and rather wrinkled, dark red
III	Prostrate habit, increased tillering, leaves and tillers very narrow, slow growing	Very tall, culms slender and weak, straw and leaf sheaths purple	Longer and very slender	Glumes stiff and keeled with secondary apical tooth	Narrow and elliptical in shape
IV	Fast growing	Taller, very stout robust straw, straw and leaf sheaths purple	Very long ($1\frac{1}{2}\times$ Holdfast), very lax and marked tapering, some basal spikelets may be replaced by branches or have supernumerary spikelets	Spikelets large and coarse, lemma points longer at apex of ear	Short and broad, wrinkled and irregular conformation, pale red

D

influence of the rye chromosomes in modifying the phenotype of wheat, and to indicate the possibilities from the direct introduction

TABLE 2

The characters in which King II rye is distinct from Holdfast wheat

	Characters in King II *
Leaves . . .	Broader and darker green with auricles which are smaller and not hairy
Stem . . .	Plants are taller, peduncles pubescent (" hairy neck "), straw purple at maturity
Ears . . .	Longer, denser, more tapering from base to apex
Apical spikelet . .	No apical spikelet differentiated in morphology and orientation
Spikelets . . .	2, rarely 3, florets fertile in each spikelet (4 in Holdfast)
Glumes . . .	Very narrow, acutely tapering, one nerve (broad, abruptly shouldered and with several nerves in Holdfast)
Lemmas . . .	Broader than glumes, long and tapering acutely to form an awn, distinctly keeled and the keel fringed with hairs
Grains . . .	Exposed in the ear at maturity, long and elliptical in shape, grey in colour (short, oval and white in Holdfast)
Diseases . . .	Susceptible to rye races and immune to wheat races of brown rust and mildew ; immune to wheat races of yellow and stem rust

* The expressions of the characters listed are in direct contrast to those in Holdfast except in descriptions where Holdfast is also described.

of rye characters the distinctions between Holdfast and King II are listed in table 2. Plants with the rye chromosomes disomic have been principally examined ; monosomics have only been considered where they differ from disomics (table 3).

TABLE 3

The phenotypic differences of monosomic from disomic additions of particular rye chromosomes to Holdfast wheat

Chromosome	Differences in monosomics
I	...
II	Leaves narrower and longer, not so erect in habit, not so fast growing, taller at maturity. Not so resistant to mildew
III	Leaves and culms broader, fewer tillers, ears broader and much more fertile
IV	No branched ears or supernumerary spikelets

The descriptions have been based on plants grown in the greenhouse, although material grown in the field was also examined. All qualitative differences were equally obvious whether the plants were from the field or greenhouse, but some of the quantitative differences were less distinct in the field. About 20 plants of each addition line were examined each year for three years, covering three generations, in the greenhouse, and a further 40 plants of each line were examined for each of two years, covering two generations, in the field. Juvenile and mature plants and ear specimens are illustrated in plate I.

The descriptions of the ears of the addition plants are based on the examination of fully ripe material. The information on disease resistance (table 4) is based on artificial infections of seedlings made by Dr R. C. F. Macer, which will be described in detail elsewhere, and observations of natural infections in the field. The pathogens used in these tests were: one wheat isolate and one rye isolate of mildew (*Erysiphe graminis*), one wheat race of yellow rust (*Puccinia glumarum*), one isolate of rye brown rust (*Puccinia dispersa*) and one of wheat brown rust (*Puccinia triticina*), and one race of stem rust (*Puccinia graminis*).

TABLE 4

The influence of individual rye chromosomes on the disease resistance of Holdfast wheat

Host	Rye pathogens		Wheat pathogens			
	Mildew	Brown rust	Mildew	Brown rust	Yellow rust	Stem rust
Rye . .	*s*	*s*	*rr*	*rr*	*rr*	*r*
Wheat . .	*rr*	*rr*	*s*	*s*	*s*	*s*
Rye-wheat amphidiploid	*rr*	*rr*	*r*	*s*	*rr*	*s*
I. Addition .	*rr*	*rr*	*s*	*s*	*s*	*s*
II. Addition .	*rr*	*rr*	*r*	*s*	*r*	*s*
III. Addition .	*rr*	*rr*	*r*	*s*	*s*	*s*
IV. Addition .	*rr*	*rr*	*s*	*s*	*s*	*s*

s = susceptible *r* = resistant *rr* = immune

Chromosome I

The leaves of plants disomic for the addition of rye chromosome I are very pale green in colour, and the early growth rate is faster than that of Holdfast. The ears considerably exceed the length of Holdfast ears, but generally have no more spikelets and so are much more lax, especially at the base. Plants with this chromosome either monosomic or disomic have pubescent peduncles, like rye, the hairs being of about the same length and density as in rye. This "hairy-neck" character is the best marker for the chromosome. The grain is similar to that of Holdfast in shape and colour, although the ends are rather more pointed. Disomic additions reacted like Holdfast to the diseases with which they were tested.

Chromosome II

Plants disomic for the addition of rye chromosome II are faster growing in the juvenile stages than Holdfast. They have a more erect habit and shorter, broader, dark green leaves which are held stiffly upright.

The ears of this addition are longer but of about the same density as those of Holdfast, and are more tapering. The apical points of the glumes are blunter than in Holdfast but the lemma points are longer especially towards the apex of the ear. Sometimes there is a slight downiness of the peduncle in this addition, but the expression of the character is irregular, so that hairy and glabrous peduncles may

occur on different ears of the same plant. The grain is smaller and of less regular conformation than that of the wheat parent, and is of a dark red colour which contrasts strongly with the pale colouring of Holdfast.

Additions of II are resistant to yellow rust of wheat and very resistant to wheat mildew, but like Holdfast they are susceptible to wheat stem rust and brown rust. However, no susceptibility to rye mildew or brown rust is introduced.

Chromosome III

Disomic additions of rye chromosome III have a very prostrate juvenile habit and a marked increase in tillering compared with Holdfast. However, they are most readily distinguished at this time by their extremely narrow leaves which are only about half the width of the leaves of Holdfast. The culms are also very slender and weak, although the leaves and culms of monosomic additions are not so narrow as in disomic additions. Chromosome III additions are late maturing and at maturity are very tall, being about one and a quarter times the height of Holdfast. When the ears are ripe the straw is dark red, due to anthocyanin deposits.

The ears of disomic additions, which are longer than those of Holdfast, are very slender partly as a result of the extremely low fertility, for the spikelets are not inflated as in a normal spikelet by the swelling of grain. Monosomic additions have broader ears than disomic additions, for in monosomic additions fertility is almost normal. The glumes are very stiff in disomics due to the presence of a pronounced keel, and they are longer and narrower than in Holdfast, with a narrow shoulder raised into a secondary apical tooth. The grains are narrow and elliptical in shape.

The most marked influence of this chromosome is in reducing the width and to some extent increasing the length of many organs, so that it apparently carries genes which profoundly influence the pattern of development in most parts of the organism.

The disease reactions of the chromosome III additions differ from the reactions of Holdfast only in the occurrence of very considerable resistance to wheat mildew.

Chromosome IV

Mature plants disomic for the addition of rye chromosome IV are a good deal taller than Holdfast and have very robust straw, but there are no other vegetative differences.

The ears are much longer than the ears of Holdfast, in addition they taper considerably and are very lax, especially at the base. Some of the basal spikelets are often replaced by short branches which may themselves have as many as six fertile spikelets. Branches are not developed on all the ears of a plant, and some plants may not have any, or may instead have supernumerary spikelets. That is, two spikelets may be produced one above the other at the same node, and this may happen at a number of the lower nodes of the ear. Neither supernumerary spikelets nor branches have been observed in the ears of monosomic additions. Normal spikelets in these additions are larger than in Holdfast and this, combined with the marked laxness, gives the ears a coarse appearance. The grains are shorter and flatter, and are incompletely filled and so more irregular in conformation than the grains of Holdfast. Whilst the grain is red in colour, the pigmentation is not developed to the extent that it is in chromosome II additions.

This addition does not differ from the wheat parent in disease resistance.

It should be emphasised that these addition lines were derived from one amphidiploid, which arose from one rye gamete. Thus only one genetic arrangement of each chromosome has been sampled from a population which, in an outbreeding species such as rye, may be expected to vary considerably. Therefore other addition lines founded on rye chromosomes homologous with those added in the

present work may be phenotypically different. However, the basic genetic architecture of each representative of a particular rye chromosome must be similar, and each must make a constant and characteristic contribution to the fundamental phenotype of rye. So that it is unlikely that there would be great differences between separate addition lines for homologous rye chromosomes, against the same wheat background.

Most modifications of the wheat phenotype derive from interactions between wheat genes and rye genes. Differences between the genotypes of different recipient varieties, therefore, may alter the phenotypes of addition lines founded on them. Again, however, such variation between lines may be of a minor nature since the overall genotype of different wheat varieties must be very similar, for all produce the phenotype called *T. vulgare*.

Nevertheless the variations amongst rye addition lines due to the combination of different rye chromosomes with a range of different recipient genotypes may provide an array of material of interest to the plant breeder.

5. THE MORPHOLOGY OF THE ADDED RYE CHROMOSOME

The morphology of the particular rye chromosome present in each addition line is best observed at first anaphase of meiosis in monosomic additions. Since at meiosis in monosomics the rye chromosome is a univalent, and invariably lags at first anaphase. Subsequently, when the wheat chromosomes are grouped at the poles, the rye chromosome divides into its chromatid halves to reveal the structure of each chromatid stretched by the forces of movement. Detailed comparisons of the structure of chromosomes cannot be made on this evidence because stretching tensions may vary, but general comparisons seem valid. Some of the structure of the alien univalent may also be seen at first metaphase of meiosis in monosomic additions.

TABLE 5

The morphology of the added rye chromosome

Chromosome	Description
I	There is one short arm and one extremely long arm with a secondary constriction, best seen at M1, mid-way along it
II	One arm is more than twice the length of the other. The short arm has a pronounced secondary constriction which marks off a large satellite
III	Both arms are of more or less equal lengths
IV	The arms are of unequal lengths and the shorter one carries a small terminal satellite. IV can be distinguished from II by the greater length of the short arm relative to the long arm, and by the smallness of the satellite

Each of the four rye chromosomes separately added to wheat may be readily distinguished from the others morphologically (plate II, table 5). Although chromosomes II and IV are similar in having

unequal arms and satellites on their shorter arms they are readily distinguished because of the smallness of the satellite of IV, and the greater length of the long relative to the short arm of II. Some preparations of somatic nuclei of rye (plate II, fig. 6) show one pair of chromosomes with large satellites, presumably chromosome II, and two pairs with small satellites, one of these being chromosome IV. The small satellites are not always readily seen in mitotic preparations, but Levan (1942) was sometimes able to reveal three, or rarely four, satellited chromosomes in haploid rye. The small satellites may well be the larger terminal knob formations described from rye pachytene by Lima-de-Faria (1952).

Chromosome III, with roughly equal arms, cannot be related to any particular pair of the three with this structure which may be seen at mitotic metaphases.

Rye chromosome I which O'Mara (1951) added to wheat had at first metaphase of meiosis a median constriction in the form of an isthmus separating equal arms. However, the appearance of the chromosome at first anaphase of meiosis, and the nature of its telocentric derivatives, demonstrated that the centromere was in fact distal and the arms of very unequal lengths. The median constriction obvious at first metaphase was a secondary constriction, and was still apparent in telocentrics of the long arm. O'Mara demonstrated, from plants with telocentrics of each arm separately added, that a gene, or gene complex, for the hairy-neck character of rye was carried on the long arm of chromosome I.

O'Mara's observations on the morphology of chromosome I and, repeating the use of plants with telocentrics, the location of the hairy-neck gene have been confirmed in detail in the present material (plate II, fig. 1). There can be no doubt therefore that such a chromosome can be obtained from rye, but it is curiously difficult to demonstrate a chromosome with the morphology of I at mitotic metaphase in rye. This may be because, as at first metaphase in monosomic additions, the more or less median secondary constriction is prominent, whereas the distal centromere constriction is inconspicuous. However, a heterobrachial chromosome, not unlike chromosome I has been described from pachytene studies by Lima-de-Faria (1952), although it is not apparent from this that there is a median secondary constriction in the long arm.

From the four examples described it is evident that the morphology of the rye chromosome, as seen in monosomic additions, is a useful aid in the separation and isolation of addition lines. Thus genetic effects may be related to particular rye chromosomes, so that some analysis of the gene content of individual rye chromosomes becomes possible.

6. DISCUSSION

Wheat-rye derivatives are probably as amenable for the study of alien chomosome transfer as any other plant material, and it is a

straightforward matter to add single pairs of rye chromosomes to the full complement of wheat. Each of the four rye chromosomes so added produces a characteristic alteration to the phenotype of wheat. The additional chromosome may therefore be recognised by its phenotypic effect, but it can also be distinguished by the morphology of the chromosome as seen at first anaphase of meiosis in monosomic addition.

Most of the phenotypic modifications caused by the rye chromosomes are of a quantitative nature constituting alterations to the size or rate of development of organs. There are, however, a few qualitative effects resulting in expressions entirely novel to the recipient variety. Amongst these only one, the introduction of the "hairy-neck" character, results in the development of one of the diagnostic characters which principally distinguish rye from wheat. None of the other characters which are taxonomically important in rye—the very narrow single-nerved glume, the long hairs on the keel of the lemma, exposed grain and the absence of a distinctive terminal spikelet—are introduced by the rye chromosomes examined. It seems probable that the non-hairiness of the peduncle of wheat is subject to no positive genetic control, so that in the presence of a rye gene, or gene complex, promoting the development of hairs, there are no antagonistic wheat genes. However, the other diagnostic characters are probably subject to direct control in wheat. For it is reasonable to suppose that the wheat genotype is organised to control the form and orientation of the terminal spikelet and the width of the glume, and so on. Further, the effective wheat genes, together with their intact arrays of modifiers, are epistatic to the rye genes, on isolated chromosomes, controlling the alternative expression of the character.

Other qualitative modifications of the phenotype of Holdfast are also due to the direct transfer of characteristics of rye. These are the introduction of resistance to wheat mildew, by chromosomes II and III, and wheat yellow rust by chromosome II ; and the introduction of the purple straw character by chromosomes III and IV.

The presence of at least two genes which cause the development of resistance to wheat mildew calls for comment. Rye is immune to wheat mildew, but the genetic system controlling immunity is incompletely epistatic to that responsible for susceptibility in wheat, since the wheat-rye amphidiploid shows resistance, not immunity. The display of resistance rather than immunity by the addition lines, therefore, need not be interpreted as evidence of the additive effects of the two genes to produce immunity in rye. Although the simultaneous selection of both genes is readily comprehensible on such a hypothesis.

If, however, each gene alone could produce immunity, the ultimate phenotype, in rye, then the problem of their simultaneous selection and fixation in the same genotype is raised. This situation may have arisen either by the two genes having originated in isolation and

been brought together by crossing, or from the simultaneous selection of both genes due to their conferring resistance to different, though overlapping, ranges of races of the pathogen. Alternatively one, or both, of the genetic systems which confer mildew resistance may not be favoured by natural selection, in rye, for its disease resistance properties. The resistance which is displayed in the addition lines may merely be a side-effect of the modification of some synthesis not directly related, through selection, to disease resistance. Indeed the two host species may have diverged to such an extent over the whole range of their physiology that a single form of the pathogen is no longer sufficiently flexible to attack both. Specialisation of the pathogen might then preclude the possibility of its attacking the alternative host, so there would be no selection for resistance. No specialised system of resistance could therefore be transferred with any rye chromosome, but the modification of physiological processes might lead fortuitously to the development of resistance in addition lines.

Amongst the qualitative effects derived from interactions between rye and wheat genes are the development of red grains by chromosome II and IV additions, and the occurrence of branched ears, or supernumerary spikelets, in chromosome IV additions. The latter is particularly interesting because of its variable expression, occurring as it does in a genetically uniform addition line. All the ears of an individual are never similarly affected by branching or supernumerary formation, and the extent to which branches or supernumeraries are developed varies from plant to plant, and from year to year. The end-product of the genetic system responsible for the ear type in chromosome IV additions is thus apparently extremely sensitive to environmental variation.

This resembles the behaviour of the F_1 in a cross between forms of *Triticum vulgare* with normal ears and *Triticum turgidum* with branched ears, described by Sharman (1944). When given short days and low temperature *T. vulgare* could develop branched ears, whereas given long days and high temperatures *T. turgidum* could develop normal heads. However, over the great range of sowing conditions suitable to either parent both were developmentally stable. The species hybrid varied between individuals and between different ears of the same individual when subjected to a range of normal sowing conditions. Thus the developmental homeostasis of *T. vulgare* and *T. turgidum* depended on the genetical determination of the threshold of physiological response, but the threshold of each was at opposite extremes of the environmental range. In the F_1 the point of response became intermediate in the environmental range so that developmental stability in normal environments was lost. The genetic control of stability in normal environments had probably, as Mather (1953) has proposed, been built into the parental genotypes by selection, very likely through the selection of polygenes. Certainly the control

of stability of the hybrid was disturbed in a way easily understood in terms of the intermediate expression in F_1's, of characters dependent on many genes. It seems reasonable, incidentally, to refer to variations in ear type as developmental instability (Mather, 1953) that is non-adaptive, rather than developmental flexibility (Thoday, 1953) that is adaptive, since the latter implies exposure to selection in environments at which the different expressions of the character are produced.

The situation in rye chromosome IV addition lines differs from that in Sharman's wheat hybrid in that neither parent of the addition derivative exhibited the branching character. The novel genetic system causing the expression must therefore depend on interactions which are environmentally sensitive. The instability of a character dependent on the interaction of wheat and rye genes can be understood in relation to the instability of organisms in which the selected gene-balance has been disorganised by interference with the breeding system (Mather, 1953 ; Jinks and Mather, 1955), or by wide crossing as in the *T. vulgare* × *T. turgidum* example. The distinction being that instead of disrupting a genotype already adjusted by natural selection to maintain developmental stability, in the addition line there had been no opportunity for selection to make the adjustments. This resembles the instability of characters dependent on newly arisen mutants (Waddington, 1942).

The most marked effects of the disomic addition of rye chromosomes to wheat is on quantitative characters such as are normally considered to be subject to the control of many genes. Each of the four addition lines differs from wheat in height and straw thickness, in the size and density of ears, in the shape of spikelets and grains, and some lines in the breadth and length of leaves. Moreover the addition lines also differ from each other in these ways. Each chromosome then produces a distinctive change in quantitative characters, and the changes produced by different chromosomes are not always in the same direction. Thus the leaves of II additions are much broader and shorter than those of Holdfast, whereas the leaves of III additions are considerably longer and narrower than those of Holdfast. The genes causing the modification in the size of organs, no doubt in interaction with wheat genes, are not therefore distributed at random amongst the rye chromosomes.

The presence of some rye chromosomes produces changes in a similar direction in a wide range of characters, as in the narrowing of all the organs in III additions, whereas other chromosomes alter the dimensions of only a limited number of organs, as in the increased width of leaves and culms only in addition II, and the increase in culm width only in IV additions. Thus one rye chromosome has genes which produce similar quantitative effects throughout the plant, whereas genes in other chromosomes operate only in particular organs. A striking similarity in effect of each of the four chromosomes added is in the laxness of the ears of each addition line. Internode

length in the ears is thus increased, although the internode length in rye ears is considerably less than that of Holdfast ears. Therefore neither the rye genes nor the wheat genes, responsible for ear density, produce their normal effects.

The sum of the quantitative and qualitative effects of each rye chromosome results in a marked modification of the phenotype of the wheat to which they have been separately added. It is perhaps surprising that an additional rye pair can produce such an effect in the presence of 21 pairs of wheat chromosomes. However, each rye pair should probably be regarded as a seventh of a completely autonomous genotype, rather than the strict equivalent of a wheat pair. Further, the degree of alteration no doubt reflects the delicate balance attained in the wheat genotype in its selective adjustment to produce a particular phenotype. The phenotypic modifications resulting from the addition of alien chromosomes make study of this type of material potentially valuable in plant breeding, especially since it is possible to introduce such characters as disease resistance. Nevertheless, the value of this material to the plant breeder depends not only on the genetic variation made available but also on its fertility and genetic stability. These aspects of the behaviour of the present material will be considered subsequently.

7. SUMMARY

1. Four pairs of rye chromosomes were separately added to the full complement of 42 wheat chromosomes to produce 44-chromosome addition lines. The single pair transfers were made by a sequence of crosses and selfings in the derivatives of wheat-rye hybrids. Such transfers are required if rye characters are to be incorporated in wheat since there is no pairing between the chromosomes of rye and wheat.

2. Each rye pair produces a marked and distinctive modification of the phenotype of the wheat variety to which it is added. Most modifications are in quantitative characters, although there are qualitative changes some of which, such as the introduction of disease resistance, are potentially valuable in wheat improvement.

3. Different addition lines may be recognised not only by their phenotypes but also by the morphology of the rye chromosome as seen at first anaphase of meiosis in monosomic additions.

4. The isolation of single chromosomes of rye against the wheat genotype provides genetical information about rye, and enables genetic effects to be related to particular chromosomes, in a way not possible working with euploids.

Acknowledgment.—It is a pleasure to acknowledge the interest and encouragement of Dr G. D. H. Bell in the course of this work.

Plate I

FIG. 1.—Mature plants of, from the left, Holdfast, and disomic additions of chromosomes I, II, III and IV.

FIG. 2.—Juvenile plants of, from the left, Holdfast, and disomic additions of chromosomes I, II and III.

FIG. 3.—Ripe ears of, from the left, King II rye, Holdfast, and disomic additions of chromosomes I, II, III and IV.

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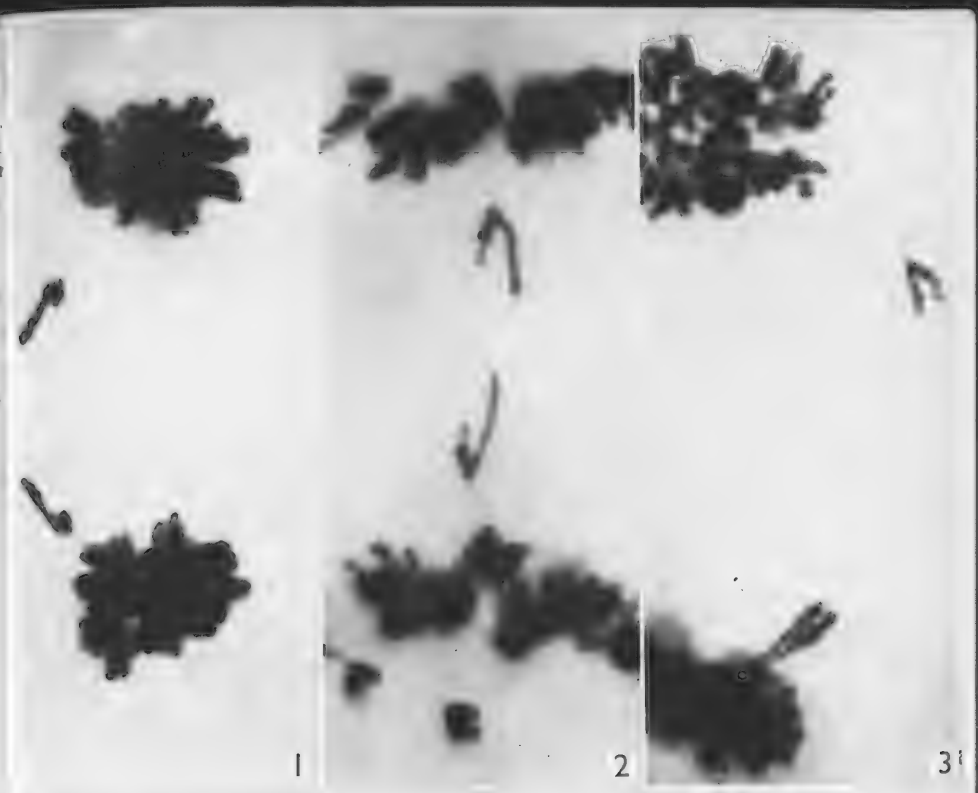
Plate II

FIG. 1.—Chromosome I. FIG. 2.—Chromosome II. FIG. 3.—Chromosome III. FIG. 4.—Chromosome IV, shown as univalents dividing into their chromatid halves at first anaphase of meiosis in 43-chromosome monosomic addition plants. ×2200.

FIG. 5.—Mitotic metaphase from a root-tip of King II rye fixed after 24 hours at 0-2° C. ×2600.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GENE-ENZYME RELATIONSHIPS AT THE *am* LOCUS IN *NEUROSPORA CRASSA*

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1. INTRODUCTION

THE amination deficient, *am*, locus in *Neurospora crassa* is of particular interest, since previous work (Fincham, 1954; Pateman, 1957), has shown that mutation at this locus specifically affects the formation of the enzyme glutamic dehydrogenase by the organism. A number of independently induced *am* mutants are available and a genetical and biochemical investigation of four of these is described in this paper.

2. METHODS

The origin of the experimental strains has been previously described (Pateman, 1957; Fincham and Pateman, 1957*b*) with the exception of am^R which is an amination deficient mutant kindly supplied by Dr J. L. Reissig. The mutants am^{32}, am^{47} and am^1 were induced by ultraviolet light, am^{29} by 20-methylcholanthrene and am^R by β-propionolactone.

Media. For growth tests and for the keeping of stock cultures, either Fries No. 3 medium or the minimal medium "N" of Vogel and Bonner (1956) was used, with suitable supplements where necessary. Mycelium for enzyme extraction was grown on Fries No. 3 or on N-free buffered medium (Fincham, 1954) supplemented with 0·01 *M* ammonium tartrate and other supplements where necessary. All crosses between strains were made on medium favouring sexual reproduction. (Westergaard and Mitchell, 1947).

Enzyme extraction. Mycelial pads were grown in 50 ml. lots of medium in 250 or 350 ml. conical flasks or in 150 ml. of medium in penicillin flasks for 48 hr. at 25° C. from a heavy inoculum of conidia. The cultures were not agitated during growth. The pads were thoroughly washed with distilled water, blotted to remove excess moisture, ground in 3-5 times their weight of 0·05 *M* *p*H 8·0 or *p*H 7·4 phosphate buffer (KH_2PO_4—Na_2HPO_4) in a chilled mortar with powdered glass, and filtered by suction through a layer of kieselguhr after the volume of buffer had been made up to 20-30 times the weight of mycelium. The filtrates were almost clear and were used without dialysis. Protein contents of extracts were determined by the quantitative biuret procedure described elsewhere (Fincham, 1954).

Glutamic dehydrogenase assay. Glutamic dehydrogenase activities were determined in the following system: 1·0 ml. extract; 1·6 ml. 0·1 *M* phosphate buffer, *p*H 8·0 or *p*H 7·4; 0·1 ml. 0·1 or 0·2 *M* sodium α-ketoglutarate; 0·1 ml. 0·1 $N(NH_4)_2SO_4$; 0·2 ml. reduced triphosphopyridine nucleotide (TPNH) solution to give an optical density at 340 mμ of 0·3-0·4. The rate of oxidation of TPNH was followed by measuring the rate of decrease of optical density at 340 mμ in a Unicam SP. 500 spectrophotometer at room temperature (18-21° C.). The reaction was usually

started by the addition of the TPNH but, since many extracts oxidise, or otherwise destroy TPNH, at a very low rate independently of the glutamic dehydrogenase reaction, very low activities were measured as the difference between the rate before and after the addition of the ammonium salt. Specific activities are expressed as change in optical density$\times 10^3$/min./mg. protein. Specific activities obtained were considerably greater than those reported in an earlier paper (Fincham, 1954). This difference is probably due to the more nearly optimal conditions, particularly in respect of *p*H and α-ketoglutarate concentration (Fincham, 1957), used for enzyme assay in the present work.

3. WILD TYPES AND PSEUDOWILDS FROM *am*×*am* CROSSES

(i) *Crosses between* am *strains*

It has been shown previously (Pateman, 1957) that the three independently induced mutations which resulted in the strains am^{29}, am^{32} and am^{47} either occurred at the same locus or at very closely linked loci. A further series of crosses between the four strains am^{29}, am^{32}, am^{47} and am^{R} were set up comprising all six possible combinations of parents taken two at a time. In addition, crosses between identical *am* parents were up as controls. Ascospores from these crosses were spread on Fries No. 3 medium plus 1·5 per cent. agar, 1·5 per cent. sorbose, 0·75 per cent. sucrose and 0·02 *M* glycine contained in Petri dishes. The glycine strongly inhibits the growth of *am* ascospores with relatively little effect on wild-type spores (Pateman, 1957). The sorbose induces a colonial growth habit in any wild types which develop and facilitates isolation. The plates were kept at 60° C. for 35 minutes immediately after spreading and then incubated at 25° C. The total number of live ascospores was estimated by counting the germinated ascospores in twenty sample fields of each plate with a binocular dissecting microscope, after two days' incubation. The plates were examined daily and any colonies that developed were isolated and cultured; in two cases the number of colonies was large and a representative sample only was transferred. The results of these plating experiments are given in Table I.

The crosses between different *am* strains fell into two groups, which were distinct with respect to the frequency, time of development and appearance of the apparent wild-type colonies.

The apparent wild types from the $am^{29}\times am^{32}$ and $am^{32}\times am^{47}$ crosses began to form visible colonies after 5-7 days' incubation and the colonies showed great variation in the rate and compactness of growth. When tested for growth on minimal medium, the apparent wild-type isolates in this group usually gave growth intermediate between that typical for *am* and am^{+} strains. In fact, all these apparent wild types which were tested proved to be pseudowilds (see next section).

The apparent wild types from the other *am*×*am* crosses began to form visible colonies after 3-4 days' incubation; these colonies were uniform in appearance with a more vigorous, compact growth than

the great majority of the pseudowild colonies. When tested for growth on minimal medium, wild-type isolates from these crosses showed growth fully comparable to that of stock wild types. When tested they all proved to be true wild types (see next section).

The frequency of true wild types from crosses between different *am* strains is very low and in the absence of suitable markers it is uncertain whether they are due to crossing-over or some such phenomenon as "gene conversion", or perhaps a combination of both. However, it is clear that the wild types are not due to normal back-mutation or contamination, since no wild types were recovered from the control crosses between identical *am* strains.

TABLE 1

Frequency and nature of wild types from am×am *crosses*

Cross	Total no. ascospores	Total no. apparent wild types	Frequency (per cent.)	Pseudowild	True wild types
$am^{29} \times am^{29}$	585,000	0	...	0	0
$am^{32} \times am^{32}$	629,000	0	...	0	0
$am^{47} \times am^{47}$	464,000	0	...	0	0
$amR \times amR$	512,000	0	...	0	0
$am^{29} \times am^{32}$	184,000	426	*ca.* 0·23	19 *	0
$am^{29} \times am^{47}$	651,000	14	*ca.* 0·002	0	14
$am^{29} \times amR$	1,377,000	3	*ca.* 0·0002	0	3
$am^{32} \times am^{47}$	156,000	87	*ca.* 0·06	16 *	0
$am^{32} \times amR$	1,293,000	10	*ca.* 0·0008	0	10
$am^{47} \times amR$	715,000	0	<0·0002	0	0

* Only a sample of the apparent wild types was transferred from the $am^{29} \times am^{32}$ and $am^{32} \times am^{47}$ crosses.

(ii) *Nature of apparent wild types from crosses between* am *strains*

All the apparent wild types isolated were backcrossed to one of the same parental *am* strains that had been used in the cross from which they were derived. Samples of ascospores from each of these backcrosses were plated on the selective medium described above. The germinated ascospores were examined after incubation for 2-3 days at 25° C.

In those backcrosses which gave an approximate 1 : 1 segregation of wild-type and *am* ascospores, the wild-type parent was classified as a "true" wild type (see table 1) since its behaviour was similar to that of the stock wild types which carried the am^+ allele. Crosses between wild types isolated from these backcrosses and *am* strains also gave 1 : 1 segregation for *am* and am^+ ascospores.

In backcrosses of apparent wild types derived from $am^{29} \times am^{32}$ and $am^{32} \times am^{47}$ there were no am^+ ascospores in the progeny, but after about 5 days, colonies developed, similar in frequency and every other respect to those from the original $am^{29} \times am^{32}$ and $am^{32} \times am^{47}$ crosses. Since these apparent wild types did not carry the am^+ allele

and were intermediate in growth between *am* and *am*$^{+}$ strains on minimal and selective media, it seemed most probable that they were pseudowilds (Pittenger, 1954).

It is known that pseudowilds owe their origin to the presence of a disomic nucleus in an ascospore ; if the homologous chromosomes carry mutant alleles which are complementary then the resultant strain may show a semi-wild phenotype. With consequent growth and nuclear division in the pseudowild, the disomic nuclei usually break down and a heterocaryon with two types of haploid nuclei, each type carrying a different mutant allele, is formed. In order to confirm the supposition that the apparent wild types were in fact pseudowilds, it was necessary to show that they originated as single ascospores and that *am* strains of two different types could be derived from them.

This was done as follows. From $am^{29} \times am^{32}$ and $am^{32} \times am^{47}$ crosses, a number of single ascospores which showed rather strong initial growth in the selective medium, were isolated. When tested for growth on minimal and supplemented medium, cultures from these single ascospore isolates gave intermediate growth (see table 4). All the single ascospore cultures behaved like pseudowilds in back-crosses to *am* strains.

Representative strains, 1281 from $am^{29} \times am^{32}$ and 1214 from $am^{32} \times am^{47}$, were used in the following experiments. A number of single conidium isolates were made from sorbose plates streaked with conidial suspensions. All of these isolates were tested for growth on minimal medium and many behaved like typical *am* strains. A number of *am* isolates from 1281 were tested in pairs for heterocaryon formation by superimposing drops of thick conidial suspensions on glycine sorbose plates. These *am* isolates fell into two groups ; combinations involving one strain from each group gave comparatively vigorous colonies, presumably by heterocaryon formation. All the colonies which originated from mixed conidial suspension were cultured and tested for growth on minimal medium. In a similar fashion complementary heterocaryons were formed between *am* isolates from 1214. The dry weights of mycelia from tests of the *am* isolates and complementary heterocaryons in growth flasks are given in table 4. It seems clear from this evidence that the intermediate wild types from $am^{29} \times am^{32}$ and $am^{32} \times am^{47}$ were pseudowilds which gave rise to complementary heterocaryons.

It did not appear to make any difference whether the heterocaryons originated from disomic nuclei or by vegetative hyphal fusion, though pseudowild formation provided an easy, effective method of overcoming incompatibility difficulties in heterocaryon formation. The possibility remains that the semi-wild phenotype of these heterocaryons was, in fact, due wholly or in part to the presence of some disomic or diploid nuclei. This question is not settled, but evidence presented in a later section suggests that the possibility is unlikely. It is of some

interest that from no one type of cross were both true wild types and pseudowilds recovered. In those types of cross from which only true wild types were obtained, it is certain that no pseudowilds were formed, since all colonies were transferred and analysed. But it is possible that rare true wild types occurred in those crosses which produced large numbers of pseudowilds. The great majority of pseudowild and true wild-type colonies are distinct in appearance and time of development on the plates, but there is some overlap. Consequently, a rare, true wild type, which developed late, might not have been obviously different from surrounding pseudowild colonies and might not, by chance, have been included in the small sample of those transferred for testing. However, the experiments

TABLE 2

Enzyme activities of true wild types

Strains	Specific glutamic dehydrogenase activities					
14 wild types from $am^{29} \times am^{47}$ . .	410	715	505	600	550	850
	590	1062	820	320*	350*	420
	710					
3 wild types from $am^{29} \times am^{R}$ . . .	540	470	535			
10 wild types from $am^{32} \times am^{R}$ . . .	665	977	825	650	594	595
	897	472	824	768		

* Assayed at *p*H 7·4.

described and other work on the recovery of true wild types from $am^{29} \times am^{32}$ and $am^{32} \times am^{47}$ crosses (Pateman, 1956), suggests that if they occur, the frequency is at least no greater than that found for the other *am* intercrosses.

4. ENZYME ACTIVITIES OF WILD TYPES AND PSEUDOWILDS

(i) All the true wild types from $am^{29} \times am^{47}$, $am^{29} \times am^{R}$ and $am^{32} \times am^{R}$ were assayed for glutamic dehydrogenase activity. The results are given in table 2. There was some variation between the specific enzyme activities of the different wild types, but there was no evidence to suggest that any of them was significantly different from the stock wild types carrying the am^{+} allele.

(ii) A representative sample of pseudowilds from $am^{29} \times am^{32}$ and $am^{32} \times am^{47}$ were assayed for glutamic dehydrogenase activity. The results are given in table 3. There was a good deal of variation in the enzyme activities of the pseudowilds, but this was probably due to uncontrollable factors such as varying nuclear ratio. In no case, so far, has the enzyme activity of a pseudowild extract approached that of the stock wild types.

(iii) Table 4 shows the results of the enzyme assays of *am* isolates and reconstituted heterocaryons derived from 1281 and 1214, which are single ascospore pseudowilds from $am^{29} \times am^{32}$ and $am^{32} \times am^{47}$ respectively. These results illustrate the remarkable fact that certain *am* alleles, individually incapable of detectable glutamic dehydrogenase activity, are complementary in function in heterocaryons with the resultant production of enzyme. Moreover, it seems probable that this complementary action is between alleles in different nuclei in the heterocaryon.

TABLE 3

Growth on minimal medium and enzyme activities of pseudowilds

Strains		Dry weight of mycelium in mg.		Specific glutamic dehydrogenase activity
		Fries minimal	Fries plus M/200 DL-alanine	
1348 am^+		43	42	325 *
Pseudowilds from $am^{29} \times am^{32}$	1279	28	36	49 *
	1280	28	31	77 *
	1281	26	34	40 *
	1283	29	43	57 *
	1284	31	47	97 *
Pseudowilds from $am^{32} \times am^{47}$	1214	3	33	60
	1215	1	28	99
	1216	9	30	67
	1217	14	30	114

Cultures for growth tests were grown from small conidial inocula for 72 hours at 25° C. in 100 ml. flasks containing 15 ml. of medium.

* Assayed at *p*H 7·4.

5. PROPERTIES OF FORCED HETEROCARYONS

In order to extend the information derived from study of pseudowild types, heterocaryons carrying various combinations of *am* alleles were synthesised. The different *am* strains were each crossed to two arginine-requiring mutants, 46004 (*arg*-1, linkage group I) and B317 (*arg*-10, linkage group VII). These mutants were supplied by Dr Dorothy Newmeyer, who has established their linkage relationships (Newmeyer, 1957). Double mutants were isolated from the crosses, and heterocaryon formation was forced by inoculating mixtures of conidia from *arg*-1 and *arg*-10 strains on to agar slants lacking arginine but supplemented with alanine. Only double mutants which formed heterocaryons readily with the original *arg*-1 and *arg*-10 strains were used for heterocaryon formation ; all were of mating type *a*.

Table 5 shows the results of enzyme assays on heterocaryons formed between strains which, individually, were incapable of forming detectable glutamic dehydrogenase. The combinations $am^{32} + am^{47}$

and $am^{32}+am^{29}$ were found to produce the enzyme though at considerably less than the usual wild-type level. All other types of heterocaryons formed no detectable glutamic dehydrogenase in repeated experiments ; the minimum activity which should have been detectable

TABLE 4

Growth on minimal medium and enzyme activities of am *isolates and reconstituted heterocaryons from pseudowilds*

Strain	Dry weight of mycelium in mg.		Specific glutamic dehydrogenase activity
	Fries minimal	Fries plus M/200 DL-alanine	
Pseudowild 1281 from $am^{29} \times am^{32}$.	26	34	40 *
am isolates from pseudowild 1281 1	0	29	0 (<3)
2	0	24	0 (<3)
3	0	23	0 (<3)
4	0	25	0 (<3)
5	0	24	0 (<3)
6	0	22	0 (<3)
Heterocaryons between *am* isolates from 1281 1+4	26	33	80
1+5	30	37	49
2+3	29	37	96
2+6	37	38	64
Pseudowild 1214 from $am^{32} \times am^{47}$.	3	33	60
am isolates from pseudowild 1214 1	0	30	0 (<3)
2	0	34	0 (<0·6)
3	0	33	0 (<2)
4	0	34	0 (<1)
Heterocaryons between *am* isolates from 1214 1+4	10	35	75
2+3	2	33	82
3+4	9	29	84
1346 am^{+}	35	33	550

* Assayed at *p*H 7·4.

in each experiment is indicated in table 5 and varied according to the protein content of the extract and the stability of the spectrophotometer at the time. These results were checked by growth experiments. The heterocaryons which produced glutamic dehydrogenase grew almost as well as the wild type on minimal medium, while those which formed no detectable glutamic dehydrogenase grew no better on minimal medium than did homocaryotic *am* strains (*i.e.* no weighable growth after 3 days at 25° C.), although they grew at approximately wild-type rates on medium supplemented with DL-alanine at $\frac{1}{2}$ mg. per ml.

The allele am^{l}, which is described in detail elsewhere (Fincham

and Pateman, 1957*b*; Fincham, 1957) was isolated as an apparent back-mutant in an experiment in which conidia of an am^{47} strain were irradiated with ultraviolet light. The available evidence (Fincham, 1957) supports the hypothesis that am^{l} produces a glutamic dehydrogenase qualitatively different from that of the wild type. Extracts of am^{l} strains have low glutamic dehydrogenase activity which can be increased to a level approaching that of wild type by warming to 35° C. or higher for a few minutes. This extra activity is slowly lost on returning the activated extract to room temperature, but can be regained on repeated warming. Treatment at 35-50° C. does not affect the activity of wild-type extracts, nor does it induce

TABLE 5

Specific glutamic dehydrogenase activities from forced heterocaryons

		arg-1		
		am^{47} (1509-78)	am^{32} (1526-78)	am^{R} (1538-78)
arg-10	am^{47} (1507-56)	0 (<2) 0 (<2)	60	0 (<3) 0 (<0·5)
	am^{32} (13)	67 *	0 (<5)	0 (<3)
	am^{29} (14)	0 (<2) 0 (<1)	46 150	0 (<2) 0 (<5)
	am^{R} (27)	0 (<1)	0 (<4) 0 (<0·3)	0 (<4) 0 (<0·5)

* Grown on Vogel and Bonner's medium "N" instead of the usual N-free buffered medium with ammonium tartrate.

The medium was supplemented with DL-alanine in all cases, either ½ mg. or 1 mg./ml. Wild type typically gave specific activities of 500 or more grown under the same conditions.

activity in extracts of other *am* mutants lacking glutamic dehydrogenase. The factor by which the activity of am^{l} extract is increased by warming varies from experiment to experiment, but has always been at least six in the present series of experiments (table 6). The variation is thought to be due to differences in room temperature; the effect of warming has generally been less at higher room temperatures at which am^{l} enzyme is probably already partly activated or rapidly becomes so on mixing with substrates.

Results of experiments on heterocaryons carrying am^{l} in combination with other *am* alleles are shown in table 6. Extracts of heterocaryons of the type $am^{l}+am^{47}$ were qualitatively similar to am^{l} homocaryons, although their activity was generally rather less. Thus there is no indication of any complementary interaction between these two alleles. On the other hand, extracts of $am^{l}+am^{32}$ heterocaryons behaved as if they contained a mixture of wild-type and

am^{1}-type glutamic dehydrogenases (Fincham, 1957); their activity before heat treatment was very much greater than that of typical

TABLE 6

Enzyme activities of heterocaryons involving am^{1}

Strain	Experiment number	Specific glutamic dehydrogenase activity	
		Extract untreated	Extract treated at 38·5° C. for 5 min.
Homocaryons			
arg-10 *am*$^{+}$ (B317-9-9) *	1	580	590
arg-1 *am*^{1} (1519-34) *	1	30	320
arg-1 *am*^{1} (1517-56) *	1	39	420
albino am^{1} (1432-78)	3	23	250
	5	36	460
	8	45	340
Heterocaryons			
arg-10 *am*$^{+}$+*arg*-1 *am*^{1}			
B317-9-9+1517-56	1	570	800
arg-10 *am*^{47}+*arg*-1 *am*^{1}			
1505-56+1517-56	1	14	200
	6	38	220
	7	24	160
1507-56+1519-34	1	20	330
	2	9	165
	5	26	330
	6	63	390
arg-10 *am*^{32}+*arg*-1 *am*^{1}			
11+1519-34	2	160	250
13+1519-34	2	205	270
	5	205	340
arg-10 *am*^{29}+*arg*-1 *um*^{1}			
14+1517-56	7	120	220
14+1519-34	3	14	50
	4	11	54
	5	84	235
		143	290
	6	100	180
		130	180
	7 †	95	240
arg-10 *am*^{R}+*arg*-1 *am*^{1}			
27+1517-56	7	20	20
	8	28	83
27+1519-34	2	36	140
	5	37	160
	6	110	330
		21	34
	7 †	60	115
	8 †	73	110

* Grown in medium supplemented with $\frac{1}{2}$ mg./ml. L-arginine hydrochloride.
† Heterocaryon made afresh from the original stocks for this experiment.

Values derived from the same experiment are more closely comparable than those from different experiments, owing to differences in room temperature.

am^{1} extracts, but their response to heat was very much less. This result was not unexpected in view of the complementary interaction already shown between *am*^{47} and *am*^{32}. More surprisingly, both *am*^{1}+*am*^{29} and *am*^{1}+*am*^{R} heterocaryons gave evidence of producing

some enzyme resembling wild-type glutamic dehydrogenase in not responding to the temperature treatment. Extracts of heterocaryons of these two types sometimes had rather low activity before warming, but in no case was the stimulation of activity by warming as great as in extracts of am^{I} or $am^{I}+am^{47}$ cultures tested in the same experiment. The rather variable behaviour of these heterocaryons in replicated experiments is unexplained ; it could perhaps be due to fluctuations in nuclear ratio. The data as a whole suggest that am^{I} is capable of interacting with either am^{32}, am^{29} or am^{R} (but not with am^{47}) to produce glutamic dehydrogenase of the normal type. There is a strong indication that the combination $am^{I}+am^{R}$ is less effective in this respect than $am^{I}+am^{32}$, with $am^{I}+am^{29}$ perhaps occupying an intermediate position. These provisional conclusions could be better tested by experiments in which nuclear ratios were controlled. It remains possible that some of the heterocaryons involving am^{I} may produce still further varieties of glutamic dehydrogenase, but the investigation of this possibility depends on the development of other methods for distinguishing types of enzyme.

6. IS THE FORMATION OF DIPLOID NUCLEI THE BASIS OF COMPLEMENTARITY IN HETEROCARYONS ?

Perhaps the most surprising aspect of the complementarity shown by certain pairs of *am* alleles is the fact that it apparently operates with the different alleles in separate nuclei. The question arises whether the interaction is actually due to the occasional spontaneous fusion of nuclei such as is known to occur in *Aspergillus nidulans* (Pontecorvo *et al.*, 1953). If diploid nuclei are responsible for the enzyme production in $am^{x}+am^{y}$ heterocaryons they must presumably be present in the mycelium in fair frequency to account for the levels of enzyme found, since a previous study (Fincham, 1954) indicated that the level of glutamic dehydrogenase in heterocaryons was not far from being proportional to the percentage of am^{+} nuclei. On the other hand, stable diploids have never been demonstrated in *Neurospora*, although it is known that they have been looked for without success by several investigators. Thus it seems probable that diploid nuclei, if they occur, are highly unstable and become readily reduced to haploids (perhaps by a process analogous to that by which a haploid heterocaryon arises from the presumably originally disomic nucleus of a pseudowild ascospore). Such diploidisation and subsequent reduction would be accompanied by free recombination of unlinked markers. To test this possibility a search was made for conidia, produced by a heterocaryon of type *arg*-1 am^{47}+*arg*-10 am^{32}, which had become homocaryotic for one or other *arg* mutant, while remaining heterocaryotic with respect to the *am* alleles. Conidia were spread on agar plates supplemented with 0·3 mg. of L-arginine hydrochloride per ml., a concentration of arginine which satisfies an arginine requirement without permitting optimal growth of *am*

conidia. A total of 102 well-grown sporelings were isolated. All except 3 were arginine-independent, and the three which required arginine all proved to be homocaryotic for *am*. A further 75 sporelings which appeared to be growing relatively poorly on the original plate were isolated. Of these 73 required arginine, and all were homocaryotic for *am*; the other two were arginine-independent and behaved as if heterocaryotic for $am^{32}+am^{47}$. Thus there was no evidence for somatic recombination such as might be expected if unstable diploid nuclei were being formed. A further possibility is that diploid nuclei are formed which are effective in promoting enzyme formation but undergo no further division; such diploid nuclei could only be detected by cytological methods which have not yet been developed.

7. DISCUSSION

There are two main points of interest in the results described. First, the complementary action of certain pairs of *am* mutants in the production of glutamic dehydrogenase. Second, the genotypic status of the different *am* mutants and what information can be deduced from them concerning the organisation of that genetic material called the *am* locus, which apparently plays some specific role in the formation of glutamic dehydrogenase in *Neurospora*. These two points are of course inter-related, but for convenience will be discussed separately.

If, as seems probable, glutamic dehydrogenase synthesis may be promoted by the joint action of two kinds of separate nuclei, neither of which is capable of forming the enzyme by itself, a chromosome-enzyme relationship of unexpected complexity seems to be indicated. There is little doubt that *Neurospora* glutamic dehydrogenase is a single enzyme; as reported elsewhere (Fincham and Pateman, 1957*a*) neither mixtures of am^{32} and am^{47} extracts nor extracts of mixtures of am^{32} and am^{47} mycelium have been found to possess any activity, so these two mutants evidently do not produce different components of a complex enzyme system. Moreover, *Neurospora* glutamic dehydrogenase has recently been purified 40-fold (Fincham, unpublished) without great loss of total activity. The data could be interpreted as suggesting that glutamic dehydrogenase is formed in a series of distinct steps under the control of different parts of the *am* locus. In the positive heterocaryons one can imagine an intermediate in enzyme synthesis diffusing from one nucleus to another, if the enzyme is synthesised in the nucleus, or from one gene product in the cytoplasm to another, if it is synthesised in the cytoplasm. The difficulty with such a picture, apart from the current trend of biochemical opinion against multi-step enzyme synthesis (see, for example, Spiegelman, with added comment by Velick, 1956), is that it provides no obvious reason why the genes concerned with the various steps should be tightly organised in a complex locus in the wild type. As an alternative we suggest that the whole *am* locus may, in the wild type, act as a

unit in producing a single product (or plasmid, in Catcheside's (1956) terminology) which passes into the cytoplasm and becomes an essential part of the glutamic dehydrogenase-forming system. In the heterocaryons nuclear products with non-overlapping defects can be imagined as becoming organised in such a way, presumably through being built in to the same structure, that they can form a synthetic functional unit. A similar suggestion has been made by Giles *et al.* (1957) for their parallel case involving adenylosuccinase formation. Such a hypothesis requires that nuclear products be incorporated at specific cytoplasmic sites with rather high efficiency, since it is otherwise difficult to explain how the complementation could work as well as it apparently does, even though the heterocaryons have not been found to approach the wild type in efficiency of enzyme production.

The present information on the physiological relationships of the *am* mutants can be represented by a linear diagram (fig. 1). Mutations

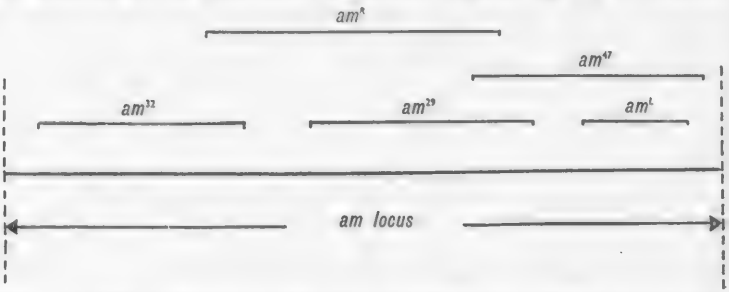

FIG. 1.—A diagrammatic representation of the physiological relationships between heteroalleles at the *am* locus.

which are mutually complementary may be envisaged as affecting non-overlapping regions of the complex locus, while mutations which are non-complementary may affect overlapping regions. Whether such a diagrammatic representation bears any relationship to the actual spatial organisation of the locus is doubtful.

Any tentative conclusions that might be made concerning the genetic status of the *am* mutants investigated, depend to a great extent on the mode of origin postulated for the wild types recovered from certain *am* intercrosses. In the absence of tetrad analysis with suitable markers, it is not possible to say whether these wild types arise as a result of orthodox crossing-over, gene conversion, or a combination of both. But it is probably significant that the wild-type frequencies from *am* intercrosses do not support any postulated linear arrangement of *am* "alleles". For example, in fig. 2 some of the wild-type frequencies from *am* intercrosses are represented in a linear diagram. This is one of the more plausible possible arrangements, yet there are two discrepancies with the rest of the data, (1) the frequency of wild types from $am^R \times am^{47}$ is less than 0·0002 per cent.; (2) there were no true wild types from $am^{32} \times am^{47}$. Furthermore, any attempt to

incorporate the data on complementarity and wild-type recovery in a single linear scheme, raises a number of anomalies. Thus it seems unlikely that all of the recovered wild types can have been the result of orthodox crossing-over, at least a proportion and possibly all of them may have been due to gene conversion. This is in line with other work on yeast (Lindegren, 1955 ; Winge, 1955 ; Roman, 1956), *Neurospora* (Mitchell, 1955*a*, *b* ; St Lawrence, 1956 ; Giles, 1951) and possibly *Aspergillus* (Pritchard, 1955). The fact that gene conversion must at least be taken into account makes it vastly more difficult to relate wild-type formation to the actual topography of the *am* locus. It has already been pointed out by St Lawrence and Bonner (1957) that the demonstration of gene conversion in organisms such as fungi, makes the interpretation of recombinant frequencies in terms of detailed spatial organisation of the genetic material a somewhat hazardous procedure. The elegant and refined analysis of the genetic material of a bacteriophage by Benzer (1957) may only be possible

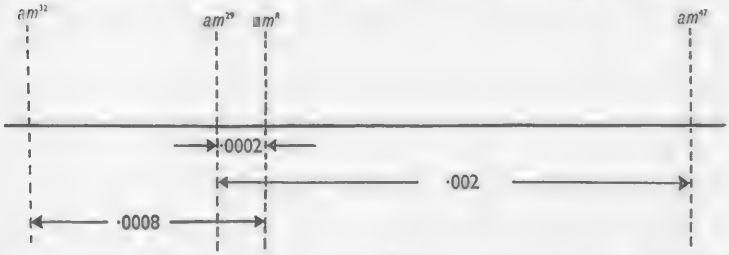

FIG. 2.—A linear diagram based on wild-type frequencies obtained from some of the *am* intercrosses.

with this type of organism and not applicable to more complex forms of life.

In order to overcome certain difficulties involved in the classical concept of the " gene ", Benzer (1957) proposed the acceptance, in its place, of three fundamental genetic units, the " recon ", the " muton" and the " cistron ". The cistron is the functional unit and two apparently similar mutants which are non-complementary in a heterocaryon are considered to be located in the same cistron. Thus am^{32} would be in a different cistron to am^{29} and am^{47}. The position of am^{R} is not so clear, since it is not complementary with am^{29}, am^{32} or am^{47} ; it might be a deletion or defect which overlaps the other *am* sites, but this seems improbable since true wild types have been recovered from $am^{29} \times am^{R}$ and $am^{32} \times am^{R}$ crosses. The case of the *am* mutants raises questions of terminology not covered by Benzer's scheme. The entity which we have referred to in this paper as the *am* " locus " is clearly some kind of significant unit since (*a*) it is concerned as a whole with the synthesis of a single specific enzyme, (*b*) its parts are extremely closely linked (and perhaps hardly separable by orthodox crossing-over), and (*c*) it is not analysable into two or

more discrete cistrons. One may ask whether the cistron is the significant functional unit in all cases and whether some other term may not be necessary to cover such cases as the present. Until more information is available on the generality of this kind of situation, we propose to continue to use the term " locus ".

In view of these considerations and with the data at present available, it seems best to regard the different *am* mutants as carrying different " heteroalleles " (Roman, 1956, and Beadle, 1957) at the *am* locus. This terminology recognises the basic fact that the *am* mutants carry demonstrably different components of the *am* locus, between which some form of gene conversion (transmutation, Beadle, 1957) can probably occur. Whether or not crossing-over can take place between these heteroalleles has yet to be satisfactorily demonstrated. The fact that certain pairs of heteroalleles are physiologically complementary provides a further criterion for distinguishing between them. It is obvious that more experimental work requires to be done with, if possible, a further group of independently induced *am* mutants, in order to extend our understanding of the gene-enzyme relationships at the *am* locus.

8. SUMMARY

(1) It has been shown that wild types can be recovered from crosses between different *am* mutants. From certain intercrosses only true wild types with a very low frequency were obtained, while from the other crosses only pseudowilds with a comparatively high frequency were recovered.

(2) The true wild types grew normally on minimal medium and possessed glutamic dehydrogenase activity fully comparable to stock wild types. The frequencies of these wild types from the various intercrosses are not readily interpretable in terms of crossing-over within a linear arrangement of " sites ", and it is suggested that at least some of the wild types may be due to gene conversion (transmutation).

(3) The growth of pseudowilds on minimal medium was intermediate between that usual for *am* and stock wild-type strains, and they possessed low glutamic dehydrogenase activity.

(4) *am* strains with no detectable glutamic dehydrogenase activity were isolated from pseudowilds and heterocaryons were reconstituted from them. These heterocaryons were shown to possess low enzyme activity similar to the pseudowilds. Thus certain *am* heteroalleles, which individually in homocaryons are incapable of producing detectable glutamic dehydrogenase activity, are complementary in function when present in different nuclei in a heterocaryon, with the resultant production of enzyme.

(5) Forced heterocaryons were made between the various *am* mutants in all possible combinations. Pairs of heteroalleles which

had given pseudowilds in sexual crosses formed glutamic dehydrogenase in heterocaryons ; pairs of heteroalleles which had given no pseudowilds in sexual crosses formed no glutamic dehydrogenase in heterocaryons.

(6) Homocaryotic am^1 strains produce a qualitatively different glutamic dehydrogenase from the wild type, but am^1 is complementary with certain other *am* heteroalleles, since forced heterocaryons produce greater or smaller amounts of apparently normal glutamic dehydrogenase in addition to the am^1 enzyme.

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STUDIES ON *STREPTOCARPUS* Lindl.

V. SPECIATION AND GENE SYSTEMS

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1. INTRODUCTION

In previous papers the genetics and chemistry of flower colour in *Streptocarpus* (Lawrence *et al.*, 1939; Lawrence and Sturgess, 1958) and the genetics of flower pattern (Lawrence, 1958) have been described. This paper completes the survey of *Streptocarpus*, gathers up the whole of the results and discusses their meaning.

2. TAXONOMY OF SPECIES

(i) *Habit*

On the basis of habit, *Streptocarpus* Lindl. falls naturally into two perfectly distinct sub-genera (Fritsch, 1904), *Streptocarpella* which comprises the caulescent species with stems (plate I, fig. 1) and *Eustreptocarpus* * the acaulescent species without stems (plates, figs. 2-5). Fritsch further divided *Eustreptocarpus*, again on habit, into two sections, unifoliati with one leaf and rosulati with many leaves. Burtt (1939), however, has pointed out that "there does not seem to be a hard and fast line" between these two sections. From observations I have made in the course of the studies on *Streptocarpus*, it has become clear that selection can produce unifoliates having a large second leaf born opposite the first one, or even a small third leaf. Further, plants raised from seed gathered in the wild from unifoliates may develop a second leaf.

In the large-leaved unifoliates, e.g. *grandis*, *dunnii*, *wendlandii*, the additional leaves could be regarded as aberrant. In certain small-leaved "unifoliates" however, the production of a second or third leaf is characteristic, e.g. *gracilis*; in others, a second leaf is very rarely formed, e.g. *polyanthus*. It will be convenient to distinguish these small-leaved true unifoliates and near-unifoliates as "sub-unifoliates", reserving the term unifoliates for the large-leaved species. The majority of rosulates produce many leaves, e.g. *rexii*, *parviflorus*, but there are some with only 6 or 7, e.g. *meyeri*.

Oehlkers (1938, 1942) has shown that in crosses between *rexii* (rosulate), and *wendlandii* and *grandis* (unifoliates), the F_2 progeny segregate into approximately 15 rosulates to one unifoliate as if two, dominant, supplementary genes controlled the rosulate character, the double recessive being unifoliate. Clearly, if modifiers affect

* Although the current rules of nomenclature require that the typical sub-genus merely repeats the generic name, the previous practice in this series of papers is continued here.

either or both of the recessive genes, or if they differ in their degree of recessiveness, the possibility exists of unifoliates (and sub-unifoliates) producing a second leaf of sorts.

In my material the difference in leaf number between unifoliates and rosulates was obvious. There are, however, two other criteria of importance for the arguments concerning taxonomy and speciation. First, unifoliates and rosulates can be distinguished by their floral systems. The unifoliates are strong-growing plants bearing stout, many-branched inflorescences each carrying roughly 30-60 moderately large flowers (plate I, fig. 3). The sub-unifoliates are weaker-growing plants and the inflorescences are smaller and more slender but each may bear some 30-60 flowers which, in contrast to those of the large-leaved unifoliates, are small (plate I, fig. 2). The rosulates are strong-growing and, typically, bear 1-2 flowers on each inflorescence (plate II, fig. 4) though some may have up to about 12 (plate II, fig. 5). Thus, with respect to the distinction between unifoliates and sub-unifoliates on the one hand and rosulates on the other, flower number appears to be a better taxonomic criterion than leaf number, the distinction being decisive in my material.

The second criterion refers to the time of flowering of the unifoliates and sub-unifoliates. In cultivation, sub-unifoliates and rosulates flower in from 4-6 months from germination, unifoliates in from 12-18 months. A difference in flowering time of this order must be an adaptive response to a major difference in climatic conditions, as must also the difference between monocarpy and polycarpy. Field studies of the distribution of polycarpic and monocarpic species of *Streptocarpus* in relation to environment, especially climate, should prove enlightening.

In the wild the division into (1) unifoliates and sub-unifoliates, and (2) rosulates may not be absolute but it holds for the 27 species (perhaps half of all those recorded) I have grown under standard conditions of good cultivation.

(ii) *Habitat*

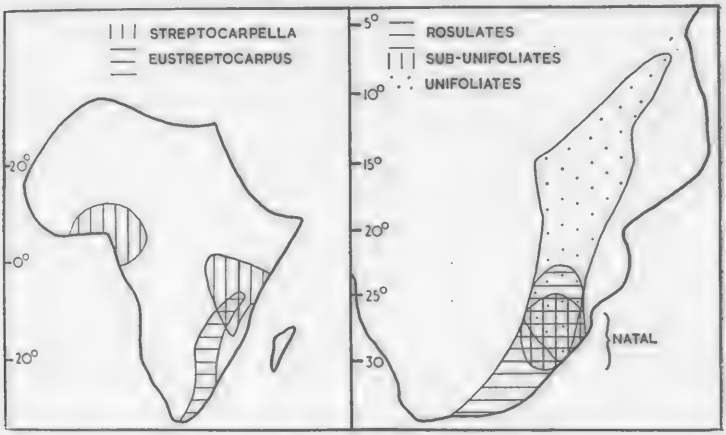
The relevance of these facts concerning habit becomes apparent when the geographical distribution of *Eustreptocarpus* is noted (text-fig.). The centre of diversity is Natal, in which unifoliates and sub-unifoliates are found together with rosulates. The sub-unifoliates known to the author occur mainly in or near Natal. Two unifoliates (*wendlandii*, *grandis*) are found in Natal, the remainder occurring northward in widely spaced areas, usually 200-300 miles apart. The rosulates overlap both unifoliates and sub-unifoliates. Of the individual species, *rexii* has the widest distribution of all, *viz.* from latitudes 25·5° to 34°. Broadly we may say the rosulates inhabit the wooded ravines and valleys of the Drakensburg mountain range and its north and south extensions and the unifoliates the more open country of the high veldt, *i.e.* habit is matched to habitat.

3. EXPERIMENTAL

The experiments described in this section all refer to *Eustreptocarpus*.

(1) *Pistil and capsule length*

Mean pistil length in the newly opened flower and mean capsule length after adequate fertilisation are given for 23 species and 4 wild varieties in table 1. The species are divided into four groups arranged in order of morphological resemblance, (1) *rexii*-like rosulates, (2) other rosulates, (3) sub-unifoliates and (4) unifoliates. On the whole, there is good correlation between the two lengths, $r = +0{\cdot}829$. The inheritance of pistil length in F_1 was recorded in 108 species crosses and the calculated mean of parental pistil length compared with the



TEXT-FIG.—*Left*: distribution of the sub-genera *Streptocarpella* and *Eustreptocarpus* in Africa. *Right*: the distribution of the three sections of *Eustreptocarpus* in south-east Africa.

observed length (to the nearest mm.) in F_1. Correlation was so good, $r = +0{\cdot}978$, that it is possible to predict F_1 pistil length accurately throughout the genus. There were no reciprocal differences. Evidently, pistil length is governed by a polygenic system, common to the genus, with no potence. Analogous phenomena have been discussed in relation to diploid species of *Berberis* (Anderson, 1953).

It will be seen, from table 1, that the four main groups of species listed there are characterised by their mean pistil lengths: *rexii*-type rosulates 37 mm., other rosulates 17 mm., sub-unifoliates 7 mm., unifoliates 26 mm. There is only one case (*montigena*) of overlapping of pistil lengths in the first three groups but group IV overlaps with groups I and II, *i.e.* it is the most heterogeneous group (this is true in a number of respects). When pistil and capsule lengths are taken together, the characterisation of the groups is even more pronounced.

Doubtless there is more variation in pistil and capsule lengths among wild plants than I have observed in my material (in several species Mr B. L. Burtt has noted capsules longer than those given above) but the mean values for the groups are sufficiently different to make it unlikely that they do not represent real distinctions.

TABLE 1

Mean pistil and capsule length in 23 species and 4 wild varieties of Streptocarpus

	Length (mm.)			Length (mm.)	
	Pistil	Capsule		Pistil	Capsule
Group I			Group III		
rexii	40	115	*pusillus*	8	14
rexii S	34	96	*daviesii*	9	35
rexii B	39	97	*polyanthus*	7	27
rexii FB	36	79	*gracilis*	7	38
rexii L	43	87	*haygarthii*	8	47
insignis	47	115	*comptonii*	5	33
polackii	33	97	*pole-evansii*	5	9
cyaneus	26	90			
gardeni	38	124	Means	7	29
Means	37	100	Group IV		
			grandis	23	43
Group II			*wendlandii*	15	72
montigena	27	64	*wilmsii*	16	52
meyeri	12	40	*dunnii*	35	64
johannis	13	47	*michelmorei*	24	53
parviflorus	17	106	*eylesii*	24	45
			vandeleuri	48	90
Means	17	64	Means	26	60

(ii) *Natural pollinations*

Although in the past twenty years I have made repeated enquiries of field botanists and others in South Africa as to the insects that visit *Streptocarpus* in the wild, no information has been forthcoming. One correspondent wrote " bees and other insects visit wide-lobed flowers in my garden ". No British insects ever visited my plants, consequently pollinations could be made without protecting the flowers and without fear of contamination.

Some evidence on natural pollination has been obtained by noting the degree to which species set seed naturally in the glasshouse (table 2). No general trends are evident with respect to the four taxonomic groups, therefore specific mechanisms concerned with pollination must come into play. For example, comparison of the relative positions of anthers and stigmas in the different species shows that proximity of these organs is often correlated with good natural

seed-setting and that species with stamens at a lower level than the stigma do not easily set seed naturally. It is clear, however, that anther and stigma positions do not account entirely for ability or inability to set seed. Pollen-tube incompatibility (p. 340) is probably a factor in some species and the highly specialised shape of the corolla tube in e.g. *polyanthus* and *johannis* strongly suggests that such species may be pollinated by long-tongued insects such as moths. Further, the flowers of *vandeleuri* from Pretoria, Transvaal and *eylesii* from Umtali, Rhodesia (600 miles distant) are identically and uniquely scented, which again suggests a specific type of insect visitor. It is unfortunate that so little is known of the pollination of *Streptocarpus*

TABLE 2

Natural seed-setting in the glasshouse

Seed-setting			
Good	Moderate	Poor	Little or none
* I *rexii* I *rexii* vars. I *rexii L* I *insignis* I *gardeni* III *pusillus* III *polyanthus* III *haygarthii* III *comptonii* III *umtaliensis* IV *wendlandii*	IV *grandis* IV *dunnii*	I *polackii* IV *michelmorei*	I *cyaneus* II *montigenus* II *meyeri* II *johannis* II *parviflorus* III *daviesii* III *gracilis* III *pole-evansii* IV *wilmsii* IV *eylesii* IV *vandeleuri*

* The numbers refer to the taxonomic groups listed in table 1.

and, therefore, so little can be said of its breeding system from direct observation. No detailed study has been made on inbreeding depression but I have observed it in a number of species (*e.g.* see p. 341). This, together with the evidence from crossability and certation (pp. 337-342) suggests that most of the *Streptocarpus* species are outbreeders.

(iii) *Interspecific crossability and fertility*

A summary of the selfing and crossing of species is presented in table 3. One hundred and ninety-one crosses were made involving 24 species, and 71 of the hybrids were selfed. In addition, 23 of the species were selfed. In most cases several flowers were pollinated in each cross or self.

Scoring was made, first on the percentage of inter- or intra-group crosses giving viable seeds in F_1 (= crossability index) and secondly on the number of seedlings per capsule from selfing, and in F_1 and F_2 (= germination or fertility index). Eight fertility classes were

used, arranged on a logarithmic basis to facilitate the distinguishing of the lower fertility classes, as follows :—

Fertilisation, or germination, failed	No. of seeds germinating per capsule						
	1-5	6-17	18-55	56-173	174-548	549-1730	1731-5480
Fertility index—0	1	2	3	4	5	6	7

Ovary length (usually 80-90 per cent. of capsule length) varies greatly from species to species but as the maximum number of seeds per millimetre of ovary length is from 20-30 in successful pollinations, restriction of seed number in species with the shorter ovaries does not affect the general assessment of fertility on the basis of the above fertility index. For example, in general, the maximum number of seeds set in species with long and short ovaries is some 2000 and 500 respectively, hence the potential seed set for nearly all species is indicated by the fertility index 6·0. Only a quarter of the possible crosses between the 24 species were made, nevertheless evidence from several different experiments show that the results may be relied upon as a broad measure of crossability and fertility.

Mean crossability and fertility indices for all combinations are 53 and 2·2 respectively. Corresponding values for rosulates crossed *inter se* are 97 and 5·2, for sub-unifoliates 46 and 1·8 and for unifoliate crosses 37 and 0·9, *i.e.* the crossability and fertility indices are much higher for rosulate than for non-rosulate crosses and highest of all for crosses between group I rosulates, *viz.* 100 and 5·8.

In both crossability and fertility, the rosulates are distinctly more effective as male parents than the unifoliates. This distinction is real, *e.g.* it can also be seen in a different series of crosses (p. 342 and table 5). Crossability and cross-fertility indices are conspicuously low in group I ×group III, *i.e.* when the species with the longest styles are pollinated by those species with the shortest styles. In the reciprocal cross, group III ×group I, the crossability and cross-fertility indices are three times as high. The same phenomenon can be seen, in decreasing degree, in group III ×group IV and group III × group II.

The percentage of F_1 plants that produced progeny from selfing was 76 and the fertility index 3·4. The corresponding figures for the rosulates were 100 and 5·6. The fertility index for F_1 plants in group I was 5·9 and for group I crossed group II, 4·9.

Thirteen reciprocal crosses failed in one direction and were successful in the other, the fertility indices ranging fairly evenly, from 0·5 to 6·0. No correlations or trends were evident, except one : 12 of the failures were from the use of species in groups III (7 failures) and IV (5 failures) as male parents, the thirteenth being a Group II

species. Thus the tendency to failure in one direction of reciprocal crossing is associated with the use of pollen from the groups with

TABLE 3

Intra- and inter-group crossability and fertility in Streptocarpus

♀ \ ♂		Group I	Group II	Group III	Group IV	Means
Rosulates						
Group I						
rexii, *rexii L*, *insignis*, *polackii*, *cyaneus*, *gardeni*	C	100/20	89/9	13/15	66/15	68/59
	F	5·8	4·4	0·5	2·8	3·5
	FF	5·3/6	...	...	...	...
Group II						
montigena, *meyeri*, *johannis*, *parviflorus*	C	100/7	100/1	43/7	33/1	62·21
	F	4·6	5·0	0·6	1·1	2·3
	FF	...	4·4/4	...	...	...
Sub-unifoliates						
Group III						
pusillus, *daviesii*, *polyanthus*, *gracilis*, *haygarthii*, *comptonii*, *pole-evansii*	C	38/8	50/4	46/13	44/11	44/36
	F	1·5	2·0	1·8	1·8	1·8
	FF	...	...	4·7/6	...	...
Unifoliates						
Group IV						
grandis, *wendlandii*, *wilmsii*, *dunnii*, *galpinii*, *michelmorei*, *eylesii*	C	72/18	50/6	29/24	37/27	44/75
	F	2·4	1·5	0·8	0·9	1·3
	FF	...	...	...	3·5/7	...
Means	C	81/53	70/20	31/59	46/59	53/191
	F	3·8	3·0	0·9	1·6	2·2
	FF	...	...	...	...	4·5/23

C = crossability index, F = cross-fertility index, FF = self-fertility index.
Figures after the oblique stroke refer to the number of crosses or selfs made.

the shortest mean style length. The significance of this is discussed in the next section.

The indices for crosses between *polyanthus*, *gracilis* and *haygarthii* reveal an affinity between these species (F_1's 4·0 to 5·0) closer than that between them and some other members of the group.

The following conclusions can be drawn from the results of species crossing :

(i) half of the species tested were crossable but mean fertility was very low.
(ii) the *rexii*-like species (group I) are highly crossable and inter-fertile and their hybrids are fertile.
(iii) the rosulates (groups I and II) are highly crossable and moderately inter-fertile, and their hybrids moderately fertile.
(iv) when crossed to sub-unifoliates and unifoliates, the rosulates are more effective as male than female parents.
(v) all other inter-group crosses show low crossability and low fertility.
(vi) in general, group crossability and fertility indices are positively correlated with the mean pistil length of the pollen parents.

(iv) *Certation*

The broad picture of crossability and fertility having been ascertained, a closer examination of the breeding system in *Streptocarpus* was made in two experiments, the first comprising simultaneous self- and cross-pollinations, the second simultaneous dual cross-pollinations.

In the first experiment, self-pollen and pollen from a different variety or species (foreign pollen) were taken in roughly equal amounts, thoroughly mixed and applied in quantity to the stigma of a flower from the plant contributing the self-pollen. The plants selected for the experiment were raised from seed collected in the wild. They ranged from closely to distantly related forms as follows. *rexii* ; *rexii S*, a white-flowered variety ; *rexii L*, a form sufficiently different from *rexii* as, perhaps, to warrant sub-specific rank ; *gardeni* and *cyaneus*, species quite distinct from one another and from *rexii* ; and *dunnii*, a large-leaved unifoliate, the other five forms being rosulates. The self-fertility indices of these six plants were 6·5, 5·2, 5·8, 4·6, 4·5 and 3·0 respectively. The total number of plants was 3885, comprising 2546 F_1 hybrids and 1339 selfs. The percentage of crossed to selfed progeny in the individual combinations is given in table 4.

Assuming that the proportion of hybrid to selfed progeny is an expression of the relative rates of pollen-tube growth, foreign pollen is seen to compete successfully against self-pollen in 20 of the 30 combinations. But the most striking result is that seen when each species is used, in turn, as pollen parent for all of the other species (columns). The success of foreign pollen is greatest with the *rexii* forms, moderate with *cyaneus*, poor with *gardeni* and least with *dunnii* (the mean value for *dunnii* is inflated by one result, viz. *rexii L* ×*dunnii*). No such gradation is to be seen when the species are considered as maternal parents (rows).

At first sight these results might be ascribed to the action of a number of genes comprising an incipient incompatibility system dependent on differential rates of pollen-tube growth, a possibility

which cannot be ruled out. For example, when single plant selection is practised, there is a fairly rapid fall-off in self-fertility, mainly in the unifoliates. Indeed, the self-fertility indices given above for the six species seem to reflect this very behaviour : *rexii* is highly fertile and the other species less so in graded order.

The self-fertility index for *dunnii* is certainly too low, and is a consequence of the inbreeding that ensues when only a small number of plants of each species can be accommodated in a glasshouse. By contrast, plants of *dunnii* from the wild have a much higher self-fertility index.

The prime cause of the graded paternal effect is undoubtedly the difference in size of the pollen grains of the different species. The greater the volume of the pollen grains, the more rapid the growth

TABLE 4

Percentage hybrids from simultaneous self- and cross-pollination

♀ \ ♂	*rexii*	*rexii S*	*rexii L*	*cyaneus*	*gardeni*	*dunnii*	Means
rexii 9700 * . .	...	87	82	63	55	19	58
rexii S, *c.* 8500 .	54	...	37	62	24	9	38
rexii L 9000 . .	89	96	...	43	22	63	73
cyaneus 7500 . .	98	89	59	...	31	14	61
gardeni 6300 . .	100	85	100	58	...	5	59
dunnii 13,000 .	100	80	78	70	4	...	81
Means . .	91	87	72	59	21	22	65

* Mean pollen-grain volume, μ^3.

of the pollen tube. Although not evident with the few species given in table 4, all of which have long pistils relative to the generic range, a survey of *Streptocarpus* species by my colleague, Dr D. Lewis, has shown that good correlation exists between pollen size and pistil length which, therefore, must be mutually adapted. Correlation between pollen size and rate of pollen-tube growth has been described in heterostyled species of *Primula*, in which " thrum " pollen is twice the volume of " pin " and grows twice as fast (Lewis, 1948).

If the results in table 4 could be attributed to pollen-grain size alone, then all the combinations below the diagonal should have a crossability index greater than 50, and in fact, all of them have but one (*dunnii* × *gardeni*). The reverse applies to combinations above the line, in which the indices should all be below 50. Six of them are not. These may be due to errors in the mixed pollination technique but it is more probable that they result from incompatibility which slows down the growth of self-pollen tubes to the advantage of the foreign pollen. In this connection, the high proportion of hybrids when *rexii* is crossed by *rexii S* and *rexii L* pollen is of special significance. The same cause would account for the success of foreign pollen in

the *dunnii* combinations (bottom row) where, on pollen size alone, self-pollen should be at a considerable advantage. It has already been pointed out that the self-fertility index for the plant of *dunnii* used in the experiment is exceptionally low, *viz.* 3·0.

Bateman (1956) has decribed cryptic incompatibility in *Cheiranthus cheiri* which closely resembles the findings in *Streptocarpus*. Artificial pollination gave a full seed set, but in the field, individual plants showed outcrossing of more than 70 per cent. Mixed pollinations showed that self-pollen, though fully capable of fertilisation, could not compete with foreign pollen which, when present in equal amounts, was responsible for over 90 per cent. of the seed set.

In the second certation experiment, pollens from two different species were applied simultaneously to the stigma of a third species and the numbers of the two different hybrids counted in F_1. All of the parental combinations in these crosses were known to yield progeny in moderate to good numbers, therefore a substantial difference in the proportions of the two hybrids in F_1 could be attributed to differential rates of pollen-tube growth (or, possibly, to competition between the fertilised ovules).

The results of the mixed cross-pollinations are given in table 5. In general, they show that :—

(1) rosulate pollen competes successfully against unifoliate pollen.
(2) pollen from a species closely related to the female parent tends to be more successful than pollen from a less closely related species.
(3) pollen from *rexii* and its varieties is universally successful.

These results from mixed cross-pollinations are in agreement with those from mixed self- and cross-pollinations and together they support the conclusions from crossability. We may conclude that two certation systems operate in the genus, the first and most important dependent on pollen-grain size, the second dependent on incompatibility between pollen and style.

An interesting parallel to the observations given in sections 6 and 7 is described in *Datura* (Buchholz *et al.*, 1935). Ten species, each with $2n = 12$ chromosomes, were crossed in all possible combinations and the percentage of normal pollen tubes which had grown at least half the length of the longest tubes was noted. Half the species had short styles, 30, 38, 40, 40 and 70 mm. in length respectively and half long styles, 120, 135, 140, 150 and 190 mm. in length. It was found that the short-styled species were each " the best cultural media for the pollens of all of the other species " and, conversely, the pollen from each of the long-styled species was the most successful in growing down the styles of the others. Essential reciprocal differences in the behaviour of the pollen tubes were not found among the short-styled species but they did occur in certain crosses between short- and long-styled species. No mention is made of pollen-grain volume.

(v) *Heterosis*

Apart from the various morphological characters employed in the identification of selfed and hybrid progenies in the experiments on certation, it was evident very early in the life of the seedlings that hybrid vigour was not only common but a conspicuous character differentiating hybrids from selfs (plate II, figs. 6-7). The comparative vigour of the different selfed and hybrid progenies was

TABLE 5

Simultaneous cross-pollinations using mixed pollens

Ref. no.	Parent species ♀	Parent species ♂	No. of plants in F_1	Ref. no.	Parent species ♀	Parent species ♂	No. of plants in F_1
1	*rexii*	*rexii S*	133	11	*rexii B*	*rexii*	226
		rexii L	169			*dunnii*	13
2	,,	*rexii S*	64	12	,,	*gardeni*	142
		cyaneus	37			*dunnii*	61
3	,,	*rexii L*	46	13	*rexii L*	*rexii B*	233
		parviflorus	46			*dunnii*	6
4	,,	*rexii B*	209	14	,,	*cyaneus*	52
		dunnii	6			*dunnii*	58
5	,,	*cyaneus*	170	15	,,	*gardeni*	218
		johannis	0			*wendlandii*	2
6	,,	*cyaneus*	440	16	*wendlandii*	*rexii*	20
		wendlandii	0			*johannis*	9
7	*rexii S*	*rexii*	57	17	*dunnii*	*rexii*	100
		rexii B	42			*michelmorei*	0
8	,,	*rexii*	135				
		cyaneus	51				
9	,,	*gardeni*	54				
		dunnii	186				
10	,,	*parviflorus*	162				
		johannis	39				

assessed by calculating the area (cm.^{2}) from the length and breadth of the first leaf of the 28-week-old seedlings, a method reasonably satisfactory for the strap-shaped leaves of young *Streptocarpus* plants. The families were raised simultaneously, under the same conditions, and scored on the same day.

First, the leaf areas of the parent species were estimated by calculating the mean for all the selfed seedlings in those families in which a given species was used as female. These means are given along the diagonal in table 6 and they show that leaf size, at the time of scoring, differed considerably among the six species, *i.e.* their rate of growth differed.

Secondly, the mode of inheritance of leaf area was examined by plotting the mean of the parent species against the mean of their hybrids, first by considering a species as maternal parent, and secondly as paternal parent, a comparison which will show up any general reciprocal difference. The summed results for the species are $r = +0{\cdot}816$, $P = 0{\cdot}001$ and $r = +0{\cdot}725$, $P = 0{\cdot}001$. The corresponding regression coefficients are $y = 2{\cdot}046x - 25{\cdot}879$ and $y = 2{\cdot}406x - 35{\cdot}583$, and the difference between them is not significant. These data show that (1) inheritance of leaf area is quantitative, (2) the behaviour of the species in the aggregate when considered alternatively as maternal and paternal parents is closely similar.

TABLE 6

Heterosis as expressed in leaf area of species hybrids

♂ /	*cyaneus*	*rexii S*	*rexii L*	*rexii*	*dunnii*	*gardeni*	Heterosis index
cyaneus	*21*	1·56	1·51	1·19	1·39	2·00	1·55
rexii S	1·28	*42*	1·60	1·69	1·40	1·59	1·53
rexii L	1·49	1·37	*53*	1·09	2·68	1·85	1·74
rexii	1·85	1·17	1·37	*61*	1·69	2·84	1·83
dunnii	1·45	1·42	2·10	1·05	*67*	1·75	1·57
gardeni	1·65	1·39	1·95	1·96	1·42	*76*	1·67
Heterosis index	1·56	1·37	1·77	1·42	1·72	2·02	1·66

italic = parental leaf area (sq. cm.)

roman = heterosis index $\left(\frac{\text{hybrid leaf area}}{\text{mean of parents}}\right)$

Thirdly, the degree of vigour, or heterosis index, was assessed by computing the ratio of mean hybrid leaf area to the mean of the parents. The heterosis indices are given in table 6 and range from 1·05 to 2·84. They show that in every cross, hybrid leaf area is greater than the parental mean although in a few cases it is obvious that it cannot be significantly greater. Individual reciprocal differences are seen to occur, the greatest being 1·69 : 1·05.

Lastly, the grand mean leaf area of all parents may be compared in the form of a ratio with that of the hybrids. The three values are, parents 1·0, species as females 1·57, species as males 1·75. Positive heterosis, therefore, predominates in these species crosses.

What is the nature of this heterosis or luxuriance? Although measurements were not made on very young seedlings, the reciprocal differences in leaf size were evident to the eye at the earliest stage that comparisons would be valid. This suggests that the heterosis observed in *Streptocarpus* results from the attainment of a high growth rate very soon after germination (Whaley, 1952), *i.e.* after a very short "lag" phase (Lewis, 1955).

Detailed information has been obtained by Beuttel (1939) who measured embryo, seed and cotyledon dimensions in *rexii*, *wendlandii*, *grandis* and their hybrids, also the rate of growth of the leaves. No heterosis was found in hybrid embryos and seeds and none worth mentioning in chlorophyll content, transpiration, percentage dry weight and ash content. Heterosis was apparent in the rate of growth of leaves and in the regenerative capacity of the hybrids when propagated from leaves. Beuttel states that the heterosis arises from the increased rate of cell division.

So far we have been speaking of inter-specific heterosis, or luxuriance. That intra-specific heterosis also occurs is shown by the inbreeding depression found in some species, e.g. *dunnii*, but the intra-specific heterosis is much less in magnitude than the inter-specific.

Finally, although the evidence from so few as six species can serve only as a pointer, it may be significant that comparison of pistil

TABLE 7

Class 1	Class 3
wendlandii	*rexii*
comptonii	*cyaneus*
	gardeni
Class 2	*parviflorus*
	pole-evansii
polyanthus	*grandis*
gracilis	*dunnii*
haygarthii	*michelmorei*
johannis	? *galpinii*

length with leaf area of the species, and with leaf area of the hybrids, suggests that these characters are positively correlated. In particular, when the *rexii*-like species are considered as female parents it will be seen that the heterosis index of their hybrids increases with increase of the leaf area of the species.

(vi) *Cytoplasmic inheritance*

Reciprocal species hybrids in *Streptocarpus* often differ quantitatively, *e.g.* in size of plant, size, shape, colour and colour pattern of flowers. One conspicuous difference involves the sexual organs: A × B is normal, B × A is male sterile. Characteristically the male sterility results from aborted anthers or their entire absence, filaments which are adnate for part of their length, often thin and coiled back and sometimes missing. In less extreme cases the anthers are reduced in size and without pollen, and separate instead of cohering. In one extreme case, *wendlandii* × *cyaneus*, the anthers were stigmoid. No attempt was made to record the minor degrees of abnormality often associated with male sterility (Oehlkers, 1938), the stamens being scored simply as normal or abnormal.

In my experiments, the species fell into three cytoplasmic classes (table 7). *wendlandii* crossed, as female parent, with the species in

class 3 gave abnormal (male sterile) F_1's, the reciprocal crosses being normal hermaphrodite. *comptonii* crossed with *rexii* and *pole-evansii* (both class 3) also gave abnormals, *i.e.* behaved the same as *wendlandii*.

The results with class 2 species are given in table 8. *johannis*, *polyanthus* and *gracilis* gave normal progeny crossed to *wendlandii*, as if they belonged to class 1 ; and the results when these species are crossed *inter se*, also with *haygarthii*, are in agreement with this view. Yet *polyanthus* and *haygarthii* when used as female parents in crosses with class 3 species, give normals. These species therefore cannot be allotted either to classes 1 or 3 and along with *johannis* and *gracilis* are considered to form the intermediate class 2. It is of interest that

TABLE 8

Occurrence of normal and abnormal anthers in species hybrids

Cyto-plasmic group	♀ \ ♂	*wend.*	*compt.*	*joh.*	*poly.*	*grac.*	*hay.*	Group 3
1	*wendlandii* . .		N	N	N	N	...	A
	comptonii . .	F		F	F	F	N	A
2	*johannis* . .	N	...	...		...	...	F
	polyanthus . .	N	F	N		N	N	N
	gracilis . .	...	F	...	N		...	F
	haygarthii . .	F	F	...	N	...		N
3	*rexii*, and other class 3 species.	N	N	N	N	F	F	N

N = normal stamens
A = abnormal stamens
F = cross failed

johannis, a rosulate, occurs in class 2, along with the three sub-unifoliates. *johannis* is almost certainly a natural hybrid between *polyanthus* and *rexii* (p. 351). As we have seen, the latter is much more likely to have been the pollen parent than *johannis* and in this case *johannis* would be expected to carry class 2 cytoplasm.

The garden forms of *Streptocarpus*, derived from artificial hybridisation of *rexii*, *parviflorus* and *dunnii* (Lawrence, 1958), should fall into class 3 since these parents are themselves in class 3, and the results from several crosses with garden forms fulfilled this expectation. 73/37 × (*rexii* × *wendlandii*) gave normals, but (*wendlandii* × *rexii*) × 73/37 gave abnormals as did also *wendlandii* when crossed by 1/33 and 59/36. Other crosses that gave normals are : 73/37 × (*rexii* × *comptonii*), *polyanthus* × 1/33, *rexii* × (*rexii* × *comptonii*), (*rexii* × *comptonii*) × *comptonii* and (*rexii* × *comptonii*) selfed. All these results are consistent with the threefold classification described above.

The behaviour of class 1 and class 3 species when crossed reciprocally

is attributable to interaction of nuclear and cytoplasmic factors such that class 1 genomes are able to function in class 3 cytoplasm to produce normal hermaphrodite plants but group 3 genomes in class 1 cytoplasm cannot. This view assumes that in fertilisation, little or no cytoplasm is contributed by the male gametes.

That the constitution of the cytoplasm is fundamental to the phenomena observed is especially evident from an F_2 raised from selfing the hybrid *wendlandii* ×*cyaneus*, a few flowers of which bore a little pollen. Three F_2 families were raised from three different pollinations and yielded 4, 5 and 5 plants respectively, all of which bore abnormal flowers, though the degree of abnormality varied. Thus, although gene segregation must have occurred, and probably accounted for the variation observed, the cytoplasm was essentially of the same constitution as in the F_1 plant.

The *wendlandii* ×*cyaneus* hybrid was also crossed as male parent to a normal garden form and gave 41 individuals. The first flower to open on each of these individuals was scored for the condition of the anthers ; 25 were found to be normal and 16 had separated, *i.e.* aberrant, anthers. Three weeks later, when the family was in full flower, only an occasional flower was found in which the anthers were separated. Thus in class 3 cytoplasm the F_1 nuclei reacted normally, though there seems to have been either a lag in the mutual adjustment of nuclei and cytoplasm or a small contribution of cytoplasm was made by the male gametes.

This same phenomenon was also observed in two plants derived from (*wendlandii* ×*gardeni*) ×*wendlandii*, which again shows that the nuclear factors play a large part in the nuclear-cytoplasmic interaction.

Oehlkers (1938), studied cytoplasmic inheritance in *Streptocarpus* in detail and found that (1) species could be grouped for their cytoplasm, (2) the expression of sex depended on both cytoplasmic and nuclear factors, and (3) there were grades of femaleness and maleness, *i.e.* deviation from the normal hermaphrodite condition in the abnormal segregates. We may note in particular that Oehlkers found *wendlandii* and *comptonii* to comprise one class and *rexii*, *grandis*, *solenanthus* and garden forms another. *polyanthus* ×*wendlandii*, the reciprocal cross, and (*polyanthus* ×*wendlandii*) ×*polyanthus* gave nothing but normals, but in the backcross (*wendlandii* ×*polyanthus*) ×*polyanthus* half the progeny were normal and half abnormal. Further, the F_2 from *wendlandii* × *polyanthus* gave three-quarters normal and one-quarter abnormal progeny. Oehlkers concludes that *polyanthus* is an intermediate form between *rexii* and *wendlandii*.

The evidence on cytoplasmic inheritance in *Streptocarpus* may be summarised as follows :—

(i) 15 species tested fell into 3 distinct classes.

(ii) the two species comprising the smallest class have the most specific cytoplasm and come from Natal (*wendlandii*,

unifoliate) and the extreme south-east Transvaal (*comptonii*, sub-unifoliate), *i.e.* in or fairly near Natal.

(iii) the species comprising class 2 are mainly sub-unifoliates and are distributed in or around Natal. The class is intermediate to classes 1 and 3.

(iv) the species comprising class 3 are either unifoliates or rosulates which are distributed from Natal to the extreme north (unifoliates) and to the extreme south (rosulates) of the *Streptocarpus* area.

4. DISCUSSION

The special aspects of *Streptocarpus* discussed in this paper fall into three categories : habit and habitat, the breeding system, speciation. To these three categories can be added a fourth, descriptive of the genus in general terms and concerned with gene systems.

(1) *Habit and habitat*

As we have seen, the unifoliates and the rosulates are two very distinct types with respect to their habit of growth. Their geographical distributions also are distinctly different, that of the unifoliates being discontinuous and northward of Natal in the high plains, while the rosulate distribution is more or less continuous and mainly southward of Natal, in shady valleys and woods. Although this correlation of habit and habitat is not absolute, without doubt it is an important criterion in considering speciation and evolution in the genus.

Oehlkers' finding that certain rosulate and unifoliate species are apparently differentiated with respect to leaf number by only two major genes is of much interest. It suggests that, first, leaf number is of major ecological importance and secondly, during speciation in *Eustreptocarpus* genes affecting leaf number were brought together (linked) in each of two groups to constitute the two postulated major genes. It also raises the question to what extent this simple Mendelian control of habit bears on the stabilisation, migration and evolution of species. An analogous example of control of different habits by a single gene has been reported by Correns (1904) for annual and biennial varieties of *Hyoscyamus niger*.

Turning to the sub-unifoliates, their distribution is striking in that they overlap the point of juncture of the unifoliates and the rosulates. Again, this criterion is not absolute but the facts are too obvious to be overlooked or lack significance. Thus the sub-unifoliates bridge the " gap " between unifoliates and rosulates on the criteria of both habit and habitat. It is not impossible that some, or all, of the sub-unifoliates also bridge this gap with respect to the genes controlling leaf number, *e.g.* rosulates *AABB*, unifoliates *aabb* and sub-unifoliates *A*(*A*)*bb* or *aaB*(*B*) plus, of course, " modifying " genes specific to a given species.

Another feature that broadly characterises the sub-unifoliates is

their cytoplasmic heredity. This time, however, the geographical pattern is not so much a linear one as with habit and habitat but roughly concentric, class 1 species being moderately near, or in, the centre, class 2 (mainly sub-unifoliates) occupying an inner ring and class 3 the outer northern and southern extensions of the *Streptocarpus* area.

Heterosis may be considered here as a component of habit. Interspecific heterosis, *i.e.* luxuriance, predominates in *Eustreptocarpus* to an extent that justifies its being described as a generic characteristic and it occurs regardless of whether the parent species are distantly or closely related. Heterosis is not found in pistil and capsule length or in pollen-grain size, *i.e.* in floral characters. Heterosis, therefore, is expressed mainly in the vegetative system, *i.e.* in the highly specialised leaf structure and growth, in which one of the sessile cotyledons dies and the other develops by proliferation of the meristematic tissue at its base into a leaf (Hill, 1938). A few leaves are later developed in the rosulates. The specialised single leaf of the unifoliates, also the relatively few leaves of the rosulates, must be adapted to equally specialised ecological factors.

The common occurrence of heterosis in *Eustreptocarpus* can be explained by postulating that each species carries a different combination of dominant genes controlling the rate of growth of the leaves, the combination in a given species serving to regulate growth so that an excessively fast or slow rate is avoided. Species crossing then results in a larger number of dominant growth genes being brought together than is found in the individual species, with a consequent enhancement of rate of growth.

It would be of interest to ascertain the comparative character of inter- and intra-specific heterosis in unifoliates, sub-unifoliates and rosulates respectively, whether heterosis is found in the caulescent species and, if so, what the relationship of the caulescent heterosis is to the acaulescent.

(ii) *The breeding system*

It has been established that a positive correlation exists between pistil length and pollen-grain size in *Streptocarpus*. The inheritance of pistil length is quantitative or polygenic, with no potence, hence, by inference, so also is the inheritance of pollen-grain size. Also, species with large pollen grains are more effective as male parents in species crossing than those with smaller pollen grains. This behaviour has also been demonstrated in mixed pollinations, *i.e.* other things being equal, large pollen grains produce the faster-growing pollen tubes.

To what extent could, or do, these factors constitute the mechanism of a generic breeding system? Broadly, selection leading to increase in pollen-grain size would seem to be an advantage for a species in which a measure of cross-pollination also was an advantage, *i.e.* pollen size may be regarded merely as one of a number of devices for promoting outbreeding. More precisely, the answer, so far as

pollen size alone is concerned, depends on the variation that occurs within species and between closely related sub-species and species. There is no evidence on the former but in *rexii*, *rexii S* and *rexii L* the differences in mean pollen sizes are not inconsiderable, *viz.* 9700, 8500 and 9000 μ^3 respectively.

The difference between *rexii* and the acyanic wild variety *rexii S* is of interest. Like many other white-flowered varieties of plants, *rexii S* is a little smaller, in many respects, than the type. The only known genetical difference between the two genotypes is that *rexii* is homozygous dominant for the gene *F* producing anthocyanin in the flowers, whereas *rexii S* is homozygous recessive. If mutation of such a gene as *F* results in a smaller plant, including smaller pollen, then, apart from other considerations, hybridisation with the type is likely to lead, through certation, to the rapid elimination of the mutant form from the population.

This concept, however, is too simple for it does not take into consideration the probable existence of a second outbreeding mechanism in *Streptocarpus*, namely, pollen-incompatibility. When *rexii* was used as female parent in mixed self- and cross-pollinations, pollen from *rexii S* and *rexii L* was distinctly more successful than self-pollen, despite the larger size of the latter, *i.e.* a degree of self-incompatibility operates to the advantage of the foreign pollen. Such is not the case when wider crosses are made, e.g. *rexii* crossed by *cyaneus* or *gardeni*. The fact that the smaller pollen of *rexii S* and *rexii L* competes successfully against pollen of *rexii* indicates that pollen size and pollen-incompatibility are independent factors in certation, at least in some cases.

Now, by inference, inheritance of pollen-grain size in *Streptocarpus* is polygenic, hence it would probably be least efficient as an outbreeding mechanism in the closest crosses, *e.g.* sib-crosses. Conversely the success of an incompatibility system is dependent on simple gene differences, *e.g.* S_1, S_2, etc., and this system would be most efficient in sib-crosses. In other words, pollen size is quantitative and incompatibility qualitative in inheritance and function and together these systems could comprise a sensitively balanced mechanism promoting outbreeding within a species and, possibly, between sub-species. Variability would be stored within the population, as well as within the individual, facilitating adaptation to new or changing environments. That *rexii* populations, even from very small areas, are heterogeneous is beyond doubt. For example, quantitative variation in flower colour and pattern is characteristic of this species and the frequency with which recessive genes have turned up in material from the wild, supports the inference from visual assessment.

This brings us to a closer examination of *rexii* in relation to speciation. The seeds of *Streptocarpus* are exceedingly small, some 50,000 per gram, and in *rexii* very numerous, probably 2000 to 20,000 per plant per season. Such small seeds would often be carried on the

feet of birds and small animals some scores of feet from the parent plant and occasionally to much greater distances. Provided the territory was suitable, colonisation would proceed rapidly. Actually, *rexii* ranges from the wooded country of the south of Cape Province, in a narrow belt across the furrowed eastern slopes of the Drakensburg mountains, into Natal, and from thence northward as far as Barberton in the south-east of the Transvaal. This extensive area can be described, broadly, as hilly and wooded, with a characteristic rainfall. Locally, this stretch of country may vary greatly, in altitude, soil type, shelter, rainfall, etc. It is obvious that this topographical variation is matched by the actual and potential genetical variation that characterises *rexii*. All other species have, by comparison, restricted geographical ranges and not infrequently they are highly restricted.

What is it that differentiates *rexii* as the most successful species? Of the various *Streptocarpus* characters studied by the author none is unique to *rexii*. On the other hand, several rare or extreme characters are combined in *rexii*. Thus, the trumpet-shaped flower with an expanded limb is only exceeded in size by *rexii L* and *insignis* among the rosulates and by *vandeleuri* among the unifoliates. All three of these species have a highly localised distribution. Pistil length in *rexii* is among the longest in *Streptocarpus*, and crossability, freedom of seed setting and germination are exceptionally high. Only *rexii L*, *insignis* and *vandeleuri* have longer pistils and these forms are less fecund than *rexii*. Of the species examined, pollen size in *rexii* is exceeded only by that of *dunnii*, but this species is confined to one area in the Transvaal. In the glasshouse, natural seed setting, in terms of the number of capsules set, is equalled by *gardeni*, *pusillus*, *polyanthus*, *haygarthii*, *comptonii* and *wendlandii* but all these species have delimited distributions. Thus, the combination of features that differentiate *rexii* from all other species refer to its floral or reproductive mechanism; large flowers and pollen grains, long pistils and capsules, and high fecundity.

Finally, although from field studies there is no direct evidence that hybridisation of species occurs in the wild, the results of hybridisation in the glasshouse lend support to the probability of its occasional occurrence. Thus, the hybrid *rexii* × *polyanthus* strikingly resembles the rosulate *johannis*, a species whose distribution (lat. 31-32°) apparently does not now overlap with that of *polyanthus* (lat. 29-30°). In the hybrid the rosulate was dominant to the sub-unifoliate habit and in this connection it will be noted that *johannis* occupies rosulate, not sub-unifoliate territory.

Another plant from Natal, presumably a natural hybrid, closely resembled the hybrid obtained in the glasshouse by crossing *gardeni* with an unnamed and very distinct species sympatric with *gardeni* and the other two forms. It may be significant that the anthers of the presumed natural hybrid carried a large percentage of bad pollen.

In speaking of crossability it must not be assumed that species

crossing in *Streptocarpus* is either common or successful in the wild. On the contrary, many species are spatially isolated and the sympatric species seem to be physiologically isolated. Similarly, it must not be assumed that hybrids and their progeny would usually survive successfully. Disharmony of genetic factors would, as a rule, lead to degeneration of hybrid progeny, especially in unifoliate crosses.

(iii) *Speciation*

Eustreptocarpus comprises species showing much morphological diversity, even within a single group, *e.g.* sub-unifoliates. In the first place, this phenotypic diversity is clearly related to diversity of habitat. The mountains, valleys, woods and high veldt of south-east Africa provide locally, many different combinations, in different degrees, of insolation, precipitation, wind velocity, temperature, soil factors, and so forth, including seasonal changes in climate.

Secondly, the different species must have colonised the various habitats by reason of the high genic variability that characterises this outbreeding genus, and which is the outcome of a degree of hybridisation. Direct evidence on the frequency of hybridisation of *Streptocarpus* in the wild and of hybrid swarms is lacking. But there is evidence that new species have arisen from *rexii* at the periphery of its area of distribution, viz. *insignis* around Port St John, C.P., and *polackii* in the Barberton region of the Transvaal. *rexii L*, a subspecies of *rexii*, also is found in a peripheral area, East London, C.P., and may well be an incipient species in the process of colonising the maritime strip.

Thirdly, *rexii* is outstanding, partly on account of its wide distribution, therefore in its ability to adapt to a large range of environmental conditions ; and partly because of its potentiality to act as universal " donor " in pollination and fertilisation. In speaking of the wide distribution of *rexii* it should not be overlooked that if it occupies territory intermediate between the climatic extremes, then this territory is the most extensive type and therefore offers the greatest potential for colonisation.

The corollary of the propositions mentioned above is that the relative rate of gene-flow within and from *rexii* is high, therefore while *rexii* will show a moderate degree of phenotypic stability it will carry a large store of genetic variability. In other words, *rexii* will exhibit a degree of stabilisation or constancy at the phenotypic level which results from, and conceals, heterogeneity at the genotypic level.

Fourthly, it is evident that evolution in *Streptocarpus* has proceeded to the point where, on the one hand, a major genetic disjunction, also almost complete spatial isolation, have been established between *Streptocarpella* ($2n = 30$) and *Eustreptocarpus* ($2n = 32$) but, on the other hand, evolution is, by inference, still actively proceeding in *Eustreptocarpus*, where at one extreme inter-specific or sub-specific

hybridisation may still occur, and at the other, specific discontinuities are clearly established.

Colonisation of new territory by *Eustreptocarpus* cannot occur in a southern or eastern direction where the sea is a barrier; nor to the west where the barrier is the arid central plateau. Only to the north is expansion possible. As we have seen, the unifoliates occupy the northern region of the *Eustreptocarpus* area, but the species usually are separated by wide distances. The future of the unifoliates therefore is problematical: they could be regarded as the vanguard of a new colonisation to the north or as species surviving at the extremity of the *Eustreptocarpus* range by close adaptation to special conditions. The sub-unifoliates could expand southwards, but only in competition with the more successful rosulates. There is no obvious barrier to their expansion northward, except that they are, in general, weak-growing species rather sharply delimited in distribution and apparently adapted to special environments.

The sub-unifoliates could, of course, be supposed to be the immediate ancestral forms from which the rosulates and unifoliates have diverged and migrated southwards and northwards respectively. This view would seem to be supported by the fact that the cytoplasmic constitution of the sub-unifoliates is different from, but compatible in degree, with that of the other two cytoplasmic groups. Even so, the potentiality for the further evolution of new forms in the sub-unifoliates would seem to be remote.

The ultimate problem is to account for the differentiation of *Streptocarpella* and *Eustreptocarpus* in form, space and time. Hill (1938) states that a number of caulescent gesneriads, e.g. *Chirita viola* Ridl., have unifoliate varieties, or develop the unifoliate character under certain environmental conditions. This suggests that the actual and potential difference between the two forms is governed by a single gene or, at most, by a simple Mendelian situation. As we have seen, such a difference distinguishes the unifoliates from the rosulates. Evidently the major morphological characters that differentiate groups of species, sub-genera and even families, may be determined by one or two genes, though, of course, such genes cannot be the causal agents for the evolution of taxa.

Hill discussed the caulescent and acaulescent phenomena in detail and concluded that the unifoliate genera and species may be considered to represent the primitive condition from which caulescent forms are derived. On the other hand, Mr B. L. Burtt kindly informs me that *Streptocarpus montanus*, an apparent rosulate which is found 20° north of the northernmost rosulate, in company with caulescent species, though not at the same general altitude, has a definite abbreviated axis with well marked leaf-scars, which suggests it is a condensed caulescent rather than an acaulescent species. In this case, it is not impossible that the caulescent form is the primitive type from which have evolved the rosulate and unifoliate habits.

If specialisation continues in the sub-unifoliates, it might well lead to an evolutionary cul-de-sac and the disappearance of the section. The unifoliates would then occupy the northern part of south-east Africa and the rosulates the southern half, with little, or perhaps no, overlapping. A similar event in the past could account for the present position of the acaulescent and caulescent sub-genera, *Streptocarpella* and *Eustreptocarpus*, i.e. expansion northwards and southwards from an ancient centre of origin and diversity with the ultimate elimination of the intermediate group. On the above postulation, an evolution sequence becomes evident. The centre of origin later becomes the centre of diversity and, still later, the centre of extinction.

(iv) *Gene systems*

In this and four previous papers, Lawrence *et al.* (1939), Lawrence (1947), Lawrence and Sturgess (1958), and Lawrence (1958), accounts have been given of the genetics and chemistry of flower colour, the genetics of flower pattern and of sub-lethality, also of such features as morphology (habit), geographical distribution, crossability, certation, heterosis and cytoplasmic inheritance displayed by the various species.

In all these investigations one and the same feature has been outstanding. The characters studied do not occur in all possible combinations, *i.e.* are not distributed at random, but fall into groups or systems. These systems characterise the genus and its divisions and are the expression of gene systems concerned with form and function.

There are six major systems, two concerned with growth and four with reproduction. The nature of these systems is summarised in table 9. The first four are described and discussed in this paper. The flower-pattern system is controlled by a supergene having four components, the dominant and recessive members of which vary from species to species. Groups of species are characterised by certain combinations of the supergene components. In the flower-colour system some ten independently inherited major genes govern anthocyanin and anthoxanthin production and modification, and these genes control a syndrome in which, by gene interaction in pigment synthesis, the production of wild-type colour (malvidin 3 : 5-dimonoside) is given a high factor of safety.

Looking at the gene systems as a whole, two aspects are of note. First, the systems not only characterise, differentially, the species, sub-genera and other taxa but are themselves characterised it seems, by a duality of function, namely the simultaneous conservation of phenotypic stability and genetic variability. Secondly, the mechanism of conservation varies from system to system. In the habit system major genes would conserve stability and modifying genes would permit variability. In the certation system pollen size would conserve stability and pollen-incompatibility variability. In the flower-pattern

Plate I

FIG. 1.—A typical acaulescent species, *Streptocarpus kirkii*.

FIG. 2.—A sub-unifoliate species, *Streptocarpus polyanthus*.

FIG. 3.—A typical large-leaved unifoliate species, *Streptocarpus dunnii*.

1 3

1

2

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Plate II

FIG. 4.—A rosulate species, *Streptocarpus rexii*, with two flowers on each inflorescence.

FIG. 5.—A rosulate species, *Streptocarpus parviflorus* with up to 12 flowers on each inflorescence.

FIG. 6.—Heterosis in the rate of growth of the leaves in *Streptocarpus*. Left, *rexii* ; Centre, *rexii* × *gardeni* ; Right, *gardeni*.

FIG. 7.—Progeny from simultaneous self- and cross-pollination in *rexii* × *gardeni*. Note heterosis in the hybrids.

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system, stability is conserved, largely at the habit-group level, by a chromosomal mechanism, the supergene, but rare mutation or crossing-over would permit variation. In the flower system phenotypic stability is secured at the generic level by a chemico-genetical mechanism but recessive mutants, by subtly affecting competition for a common substrate, maintain the genetic potential for variation.

5. CONCLUSION

The characters studied in this and in previous surveys on *Streptocarpus* are not distributed at random in the genus but occur in specific combinations or systems and characterise the taxa at various levels. Each system can be seen to have the dual function of conserving

TABLE 9

Gene systems of Streptocarpus

System	Mode of Inheritance	Characters involved
Growth		
Habit	Mendelian	Leaf no. and size
Heterosis	Quantitative	Leaf growth rate
Reproduction		
Certation	Quantitative	Pollen-tube growth rate (i) pollen-size (ii) incompatibility
Cytoplasm . . .	Quasi-Mendelian	Gynodiœcy and sex modification
Flower pattern . . .	Mendelian	Gene linkage (supergene)
Flower colour . . .	Mendelian	Gene interaction (syndrome)

phenotypic stability while permitting genotypic variability. The mechanism of conservation varies from system to system.

6. SUMMARY

1. A broad survey is made of growth habits, habitats, inter- and intra-crossability and fertility, heterosis and cytoplasmic inheritance in some 27 species of the African genus, *Streptocarpus*.

2. Habit is shown to be related to habitat, both at the sub-generic level and especially within the sub-genus, *Eustreptocarpus*, which comprises species with unifoliate, sub-unifoliate and rosulate habits, having well-defined geographical distributions. The centre of diversity is Natal where all three habit groups occur, the unifoliate species ranging northward and the rosulates mainly southward from the centre.

3. Pistil length varies from about 5 to 50 mm. in *Streptocarpus*. The inheritance of pistil length is governed by a polygenic system with no potence. The different habit groups are characterised by their mean pistil (and capsule) lengths.

4. In intergroup crosses, the crossability and fertility indices are positively correlated with the mean pistil length of the species used as pollen parents.

5. This behaviour results from pistil length being positively correlated with pollen-grain volume, the largest pollen producing the fastest-growing pollen tubes. In certain crosses the rate of pollen-tube growth appears also to be controlled by pollen-incompatibility factors.

6. Heterosis, or luxuriance, in the rate of growth of the leaves which, in *Streptocarpus* are specialised organs, is evident in almost all species crosses. Heterosis is not expressed in the floral system.

7. Fifteen species tested fell into three distinct classes for cytoplasm. Class 1 ×class 3 gave male sterile F_1's, the reciprocals being normal. Class 2 gives normal F_1's with both classes 1 and 3. Class 2 species are distributed in and around Natal and are mainly sub-unifoliates, *i.e.* habit, habitat and cytoplasm type are, largely, dependent factors.

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ADDITIONAL THREE-POINT DATA FOR LINKAGE GROUP V OF THE MOUSE

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1. INTRODUCTION

THE data reported in this paper are intended to supplement the recent interference data of Owen (1953) and Wallace (1957*a*) for linkage group V of the house mouse. Owen's experiment was for the four closely linked markers agouti (*A*), undulated (*un*), wellhaarig (*we*) and pallid (*pa*), and Wallace's experiment was for agouti, fidget (*fi*) and Danforth's short-tail (*Sd*). From these studies, and from some affinity data, Wallace (1957*b*) showed that the centromere is probably between *fi* and *Sd*.

The history of this linkage group is discussed fully in Wallace's (1957*a*) paper and it will be sufficient merely to give the order of the markers :—

Ra *kr* *A* *un* *we* *pa* *fi* *Sd*

The experiment to be discussed in this paper is for the three markers ragged (*Ra*)-agouti and wellhaarig which are situated some distance from the probable location of the centromere. It is a region of the chromosome not previously studied from the point of view of interference.

2. DESIGN

The four possible triple heterozygotes

$$\frac{Ra\ A\ +}{+\ a^t\ we}, \qquad \frac{Ra\ a^t\ we}{+\ A\ +}, \qquad \frac{Ra\ A\ we}{+\ a^t\ +}, \qquad \frac{Ra\ a^t\ +}{+\ A\ we},$$

were made up and backcrossed to *Awe* mice. The symbol a^t represents tan belly, an allele of agouti, and the three types *AA*, Aa^t and a^ta^t are readily distinguishable. Data are presented in table 1 for recombination in both sexes. The data are arranged in a 4×4 Latin square of the form

A	B	C	D
B	A	D	C
C	D	A	B
D	C	B	A

where the columns represent the modes of gamete formation, the rows correspond to the four mating types, and the letters to the four pairs of complementary genotypes. This design has been used by

Wallace (1947, 1957*a*) and Parsons (1958) in the house mouse, and by other workers in *Drosophila* and maize. It enables viability differences between different complementary pairs of genotypes to be eliminated, so removing bias from the recombination fractions.

TABLE 1

Observed data

Types of gamete formation

Triply heterozygous parent	No change				*Ra* change				*we* change				*A* change					
		♀		♂		♀		♂		♀		♂		♀		♂	♀	♂
Ra A / *a*^{t} *we*	*Ra A*	57	+	77	*Ra a*^{t} *we*	19	+	26	*Ra A we*	12	+	7	*Ra a*^{t}	1	+	2		
	a^t *we*	74	+	63	*A*	23	+	20	*a*^{t}	12	+	8	*A we*	3	+	0		
		131	+	140		42	+	46		24	+	15		4	+	2	201	+203
Ra a^t *we* / *A*	*Ra a*^{t} *we*	75	+	77	*Ra A*	20	+	19	*Ra a*^{t}	12	+	17	*Ra A we*	0	+	0		
	A	55	+	57	*a*^{t} *we*	27	+	25	*A we*	16	+	6	*a*^{t}	0	+	1		
		130	+	134		47	+	44		28	+	23		0	+	1	205	+202
Ra A we / *a*^{t}	*Ra A we*	66	+	70	*Ra a*^{t}	20	+	25	*Ra A*	17	+	16	*Ra a*^{t} *we*	2	+	0		
	a^t	78	+	72	*A we*	16	+	19	*a*^{t} *we*	10	+	6	*A*	0	+	1		
		144	+	142		36	+	44		27	+	22		2	+	1	209	+209
Ra a^t / *A we*	*Ra a*^{t}	86	+	69	*Ra A we*	9	+	22	*Ra a*^{t} *we*	13	+	10	*Ra A*	1	+	1		
	A we	55	+	56	*a*^{t}	28	+	30	*A*	15	+	16	*a*^{t} *we*	0	+	0		
		141	+	125		37	+	52		28	+	26		1	+	1	207	+204
		546	+	541		162	+	186		107	+	86		7	+	5	822	+818

3. ANALYSIS

Before estimating recombination and interference, certain standard tests for heterogeneity in the data are necessary. The tests to be used are set out fully by Parsons (1957*a*, 1958) and Wallace (1957*a*) and here it is merely proposed to give some of them very briefly with little discussion. The first is a general heterogeneity test on the 4×4 Latin square testing the agreement of observations and proportionate expectations based upon column and row totals. As the entries in the double recombinant class are very small, they are ignored for this test. For the female data $\chi^2_6 = 3{\cdot}07$ ($0{\cdot}7 < P < 0{\cdot}8$) and for the male $\chi^2_6 = 5{\cdot}10$ ($0{\cdot}5 < P < 0{\cdot}7$), thus neither set of data reveals significant heterogeneity.

It is to be expected that the members of a pair of complementary genotypes will be unequal because of viability and possibly manifestation disturbance. However, if the data are reliable, such deviations

from the expected 1 : 1 ratio within a complementary pair should not be heterogeneous among the four possible parental heterozygotes.

TABLE 2

Heterogeneity tests for members of complementary pairs

2a. Data arranged for testing

Genotypes	No change	*Ra* change	*we* change	Data arranged according to sex of parent		Total
				♂	♀	
A	*Ra A* +	134	39	33	112	94	206
	+ *a*^{t} *we*	137	52	16	94	111	205
		271	91	49	206	205	411
B	*Ra a*^{t} *we*	152	45	23	113	107	220
	+ *A* +	112	43	31	93	93	186
		264	88	54	206	200	406
C	*Ra A we*	136	31	19	99	87	186
	+ *a*^{t} +	150	58	20	110	118	228
		286	89	39	209	205	414
D	*Ra a*^{t} +	155	45	29	111	118	229
	+ *A we*	111	35	22	81	87	168
		266	80	51	192	205	397

2b. χ^2 tests from data in table *2a*

Genotypes	Contingency χ^2_1 for sex differences	P	χ^2_1 for overall viability	P	χ^2_2 for heterogeneity between members of a complementary pair	P
A	2·65	0·1<P<0·2	0·002	P>0·95	7·79	0·02<P<0·05
B	0·03	0·8<P<0·9	2·85	0·05<P<0·1	4·48	0·1<P<0·2
C	0·83	0·3<P<0·5	4·26	0·02<P<0·05	4·69	0·05<P<0·1
D	0·003	P>0·95	9·37	P<0·01	0·12	P>0·90

Furthermore, there should be no difference in the magnitude of the disturbance encountered according to the sex of the triply heterozygous parent. The procedure for carrying out such tests is described fully by Parsons (1958). In table *2a* the data are arranged in a form suitable for a series of simple χ^2 tests which are tabulated in table *2b*. The double recombinant classes are ignored.

The results of these tests show that, as expected, there are deviations from the 1 : 1 ratio expected between members of complementary pairs. Such deviations are maintained throughout the data. For the complementary pair of genotypes *Ra A* and a^t *we* there is a suspicion of heterogeneity which, in view of the homogeneity of the remainder of the data, can be regarded as due to chance. No significant sex difference is detected for any of the complementary pairs.

Single-factor ratios obtained directly by addition from the observed progeny give a crude test to detect the factors responsible for the deviations from the expected 1 : 1 ratio between members of complementary pairs (table 3). There is a non-significant deficiency of *we we* mice, and a significant deficiency of *AA* mice compared with *A* a^t. Hence there is only one factor of significantly poor viability and this effect is therefore equally distributed in each cell of the

TABLE 3

Single factor ratios

	AA	*A* a^t	*Ra* +	+ +	+ *we*	*we we*
	752	888	848	792	856	784
χ^2_1 for equality .	11·28		1·91		3·16	
P . . .	P<0·001		0·1<P<0·2		0·05<P<0·1	

4 × 4 tables. In the estimation of recombination by simple addition of the column totals the effect will cancel out.

The deficiency of *AA* mice is of interest when considered in the light of Fisher's (1949) findings that the heterozygotes $A^Y A^L$, $A^Y A$, $A^Y a^t$, $A^Y a$, $A^L A$, $A^L a^t$, $A^L a$, $A a^t$, $A a$ and $a^t a$ are equally viable. However, Fisher did not discuss the viabilities of the homozygotes, one of which is *AA*. Possibly, therefore, the homozygotes at the agouti locus are of somewhat lower viability than the heterozygotes.

4. ESTIMATION OF RECOMBINATION AND INTERFERENCE

As there is little suspicion of heterogeneity, recombination can be estimated by the simple addition of the column totals of the 4 × 4 Latin squares. Recombination values and Kosambi coefficients (Owen, 1950) for each sex are listed in table 4.

The recombination values show a sex difference for each segment. For *Ra-A*, the male recombination value exceeds that of the female, and for the *A-we* segment the female value exceeds that of the male although neither difference is significant. The values for the entire segment are, however, very close to each other. The *A-we* values are a little greater than those given by Owen (1953) but as in his data, the female recombination value is greater than the male. The only previously published data for the *Ra-A* segment is a small amount

presented by Carter and Phillips (1954). The values given in table 4 are a little smaller. Data in which the male recombination value exceeds that of the female in the house mouse are not common. Wallace obtained a larger value for males for the segment *fi-Sd* in linkage group V which was, however, not significantly different from the female. There are only three other cases where male values are greater and these are discussed by Wallace (1957*a*).

Interference is more severe in the male, and in both sexes the Kosambi coefficients are well below unity, indicating that the centromere is not within the segment *Ra-A-we*. Hence, so far as they go, the data support the location of the centromere between *fi* and *Sd*.

TABLE 4

Recombination values and Kosambi coefficients

Recombination in segment	Females	Males
Ra-A	20·56±1·41	23·35±1·48
A-we	13·87±1·21	11·12±1·10
Ra-we	32·73±1·64	33·25±1·65
Kosambi coefficients	0·456±0·172	0·354±0·157

Assuming Wallace's placing of the agouti locus, the terminus (T) and the centromere (C), the markers *Ra-A-we* give a map

	T	*Ra*	*a*^{t}	*we*	C
metrical length	9	13	15	58	
map distance	15·76	18·81	17·84	38·43	
recombination		20·56	13·87		

for females with a goodness of fit $\chi^2_3 = 7{\cdot}81$ ($0{\cdot}05<P<0{\cdot}1$). This map is constructed from Fisher's segmental functions (Fisher and Yates, 1953) and it assumes Owen's (1950) $\frac{1}{4}\chi^2_4$ distribution of intercept lengths. For the males, however, the fit is not so good and the following map gives $\chi^2_3 = 9{\cdot}17$ ($0{\cdot}02<P<0{\cdot}05$).

	T	*Ra*	*a*^{t}	*we*	C
metrical length	5	17	13	50	
map distance	8·88	24·71	14·90	31·01	
recombination		23·35	11·12		

The poorer fit for the males is not unexpected as Wallace (1957*b*) obtained a poorer fit for *a*^{t}*-fi-Sd* in this sex.

In the data for which K values have been calculated in the house mouse (Owen, 1953; Wallace, 1957*a*, and Parsons, 1958) it is noticeable that they have not exceeded unity, whereas on the basis of the $\frac{1}{4}\chi^2_4$ metric which is supported by *Drosophila* (Owen, 1948) and maize (Parsons, 1957*b*), K values should exceed unity in the centromeric regions. Wallace (1957*a*) obtained K values of 0·8493± 0·1507 in the females and 0·7113±0·2887 in the males for the triple

a^{t}*-fi-Sd* which is a centromeric region. If this tendency for a somewhat more intense degree of interference is maintained in the future, some modification of the $\frac{1}{4}\chi^2_4$ metric will be necessary for the house mouse.

5. SUMMARY

Three-point data are presented for linkage group V of the house mouse for the markers ragged, agouti and wellhaarig. It is shown that interference is reasonably intense. This is expected as the region delimited by these genes does not contain the centromere.

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NATURAL CROSSING BETWEEN HOMOSTYLE PLANTS OF *PRIMULA VULGARIS*

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1. INTRODUCTION

In the distylic *Primula vulgaris* the pin plants with long styles and low anthers, are recessive (*ss*) to the thrum plants (*Ss*), which have short styles and high anthers. The naturally occurring homostyles have long styles and high anthers and are the result of a change in the gene complex determining the heterostyly (Ernst, 1928). The changed gene complex may be considered as a gene S^h, recessive to the thrum gene *S*, and dominant to the pin gene *s*. Pollen from anthers at a given level is not inhibited in its growth on stigmas at the same level whereas it is on those at different levels, so that the homostyles are fully self-fertile. The self-compatibility of the homostyles together with the close proximity of the stigma and anthers, has led to the belief that the majority of homostyle plants are self-pollinated. Thus Crosby (1949) did not consider the possibility of crossing, in the explanation he put forward to account for the varying proportions of homostyles found in extensive counts of natural populations.

It is the purpose of this paper to present evidence in favour of the occurrence of natural crossing between the homostyle plants, and discuss its bearing on population studies.

2. THE EXTENT OF CROSS-FERTILISATION

In 1946 a number of homostyle plants were brought by Professor Sir Ronald Fisher from Sparkford, Somerset, to the Genetics Department at Cambridge, where for several years homostyles, from segregating progenies, were allowed to pollinate in open-pollination plots and their progenies grown. Assuming that the homostyles selfed, flowers taken from segregating progenies would have the genotypes S^hs or S^hS^h, according to whether pins appeared in their progenies or not. In one or two of the early years thrum plants were put in the plots. The progenies of some of the homostyles from these plots were then found to contain thrums, which could only have arisen through crossing of the thrums onto the homostyles. Now, since thrum plants were not subsequently used in any of the plots, the possible legitimate crosses involving female homostyle parents are $S^hS^h \times S^hS^h$, $S^hS^h \times S^hs$, $S^hs \times S^hS^h$ and $S^hs \times S^hs$, of which only the latter could give rise to pins. Hence, if α is the proportion of plants which are the result of

self-fertilisation, $v : 1-v$ is the proportion of the genotypes $S^hs : S^hS^h$ amongst the homostyle parents and the illegitimate cross $S^hs \times ss$ is neglected, the expected proportion of pins amongst the progeny of segregating homostyles is $p = 1/4(\alpha+v(1-\alpha))$. This decreases from $1/4$ to $v/4$ as α decreases from 1 to 0. Thus the significant deficiency of pin plants amongst the progenies of supposedly selfed homostyles provides further evidence for crossing.

If the observed frequency is a/n or γ, say, the maximum likelihood estimate for α is $(4\gamma-v)/(1-v)$ with an estimated variance of $16a(n-a)/n^3(1-v)^2$.

The proportion of pins recorded in segregating progenies for the years 1946 to 1955 is given in table 1.

TABLE 1

Year	Proportion of pins in segregating progenies	χ^2_1 for deviation from 1 : 3
1946	4/34	3·1757
1947	17/104	4·1538
1948	47/257	6·1752
1950	37/212	6·4402
1951	36/171	1·4210
1952	10/54	1·2097
1953	29/164	4·6828
1954	26/134	2·2383
1955	119/452	0·4248
	325/1582	16·7560

The χ^2 on the total, for deviation from the expected 1 : 3 ratio when $s = 1$, is highly significant with $P<0{\cdot}1$ per cent. A heterogeneity χ^2 for all the years gives 15·1232 for eight degrees of freedom, the 5 per cent. point being 15·507, whereas for the years 1946-54 only, the corresponding value is 2·3590 for seven degrees of freedom. A further test shows that the difference between 1955 and the other years is significant with $P<1$ per cent., indicating that in 1955 there was apparently no or very little crossing whereas in the previous years the proportion of crossing had been more or less uniform.

If we assume no viability disturbances, v varies slightly about $2/3$, the variation depending on the frequency of crossing. The proportion of segregating progenies recorded, however, shows no significant deviation from $2/3$, and, taking v at this value, the estimated proportion of crossing, derived from the years 1946-54 only, is 82·24 per cent. with a standard error of 17·67 per cent. The effect of assuming a lower viability for the homozygous homostyles (S^hS^h) relative to the heterozygous homostyles (S^hs), as Crosby proposes, is to increase the proportion of pins to homostyles. Thus, the lower the viability assumed for the S^hS^h homostyles, the higher will be the estimated proportion of crossing. When this viability is 75·63 per cent. the estimated amount of crossing is 100 per cent. and the data will not

tolerate a value lower than this. Allowing for any illegitimate crosses $S^hs \times ss$ has a similar effect.

An obvious possible explanation for the deficiency of pins is that they have a lower viability relative to all other forms. On this assumption the years 1946-54 give an estimate of 66·88 per cent. for the relative viability of the pins, with an estimated standard error of 5·15 per cent. However, in the years 1956 and 1957, only progenies from isolated homostyles, forced to self-fertilise, have been grown. The two years show no heterogeneity and give 130/520 for the total proportion of pins from segregating progenies. The contingency χ^2 testing the difference between this proportion and that observed for the years 1946-54 is 10·2 for one degree of freedom, giving a probability less than 0·5 per cent. Since, apart from the isolation of the parent homostyles, these later progenies have been grown in conditions as similar to those of previous years as the experimental controls will allow, this difference can only be attributed to the enforced self-fertilisation of the parent homostyles.

3. A POSSIBLE REASON FOR CROSSING

In 1957 a further experiment was set up, in order to investigate the reasons previously put forward for the self-fertilisation of the homostyles, *viz.* the close proximity of stigma and anthers and the early dehiscence of the latter.

A number of plants were chosen at random from ten of the progenies and individual flowers on each plant marked with coloured thread when still in bud. From the time when the flower had first opened sufficiently for the stigma to be easily visible, the difference in height between the stigma and the anthers was recorded each day for about six days, according to the scoring system indicated in fig. 1. Scores of 0, 1, 2, 3 or 4 were assigned to flowers as the position of the stigma varied from below the anthers to well above them. The state of the anthers, *i.e.* whether they had dehisced or not, was also recorded each day. This comparatively rough scoring system was adopted in order not to damage the flowers when recording and so disturb the natural progress of their growth.

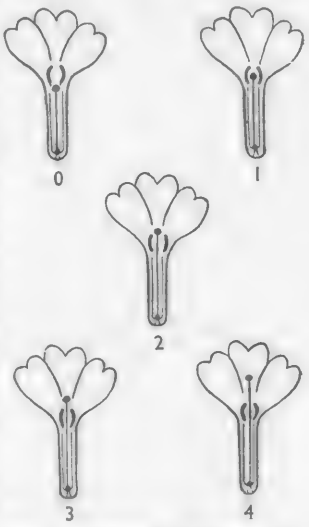

FIG. 1—Diagrams of half-flowers of *P. vulgaris*, showing typical examples from each of the scoring categories 0 to 4.

It was found that, in general, when the flower was first scored, the stigma was well above or just on top of the anthers, and that as the

corolla grew, the difference in height decreased until the stigma was below the anthers. In a number of cases the stigma actually protruded

TABLE 2

Distribution of scores for total change in relative height of stigma and anthers

Score	Progeny no.										Total
	24	23	11	12	13	14	17	20	19	15	
0	3	1	6	6	0	1	3	4	2	2	28
1	8	5	9	10	3	6	12	10	7	11	81
2	3	12	6	11	11	9	8	14	7	14	95
3	1	5	10	3	10	7	4	1	7	9	57
4	0	0	1	0	0	2	0	0	0	3	6
Total	15	23	32	30	24	25	27	29	23	39	267

Heterogeneity $\chi^2 = 46{\cdot}6282$ for 27 degrees of freedom
$P \approx 1$ per cent.

out of the bud. The overall average number of days from first recording a flower to dehiscence of its anthers was 2·75, and the flowers were usually fully open before dehiscence had occurred. The

TABLE 3

Distribution of number of days to dehiscence

No. of days	Progeny no.										Total
	24	23	11	12	13	14	17	20	19	15	
0	1	1	1	1	0	0	0	1	0	0	5
1	2	4	6	8	2	2	6	5	4	1	40
2	6	5	5	6	9	7	6	9	5	9	67
3	5	7	8	8	5	14	9	9	3	8	76
4	0	4	10	7	7	2	4	5	6	17	62
5	1	2	2	0	1	0	2	0	5	4	17
Total	15	23	32	30	24	25	27	29	23	39	267

Heterogeneity $\chi^2 = 47{\cdot}4841$ for 27 degrees of freedom
$P \approx 1$ per cent.

stigmas of forty buds, arbitrarily selected, and whose anthers had not yet dehisced, were all found to be receptive to pollen. Hence we have here a situation well known to favour outcrossing in many species.

The distributions of the scores for the total change in relative height of stigma and anthers (as measured by the difference between the initial and final scores recorded), the final recorded scores, and the number of days from first recording a flower to dehiscence of its anthers, are given in tables 2-4. In the tables, the results from plants within progenies are combined.

There is clearly considerable variation in all these characters. A contingency χ^2 calculated from the tables indicates whether the difference between the progeny distributions is significant, and thus

TABLE 4

Distribution of final score

Score	Progeny no.										Total
	24	23	11	12	13	14	17	20	19	15	
0	13	20	30	25	16	17	21	22	17	37	218
1	2	3	2	5	7	8	5	6	5	2	45
>1	0	0	0	0	1	0	1	1	1	0	4
Total	15	23	32	30	24	25	27	29	23	39	267

Heterogeneity $\chi^2 = 19{\cdot}5635$ for 9 degrees of freedom
$P \approx 2$ per cent.

whether there is any evidence for polygenic inheritance of the characters. The χ^2 were calculated on grouping the last two rows in tables 2 and 4 and also the first two in table 3. This grouping decreases the sensitivity of the test, and so there can be no doubt of the highly significant heterogeneity indicating polygenic control of the characters.

TABLE 5

Analysis of variance for total growth

	d.f.	S.S.	M.S.
Between progenies	9	29·0225	3·2247
Between plants within progenies .	46	30·4845	0·7627
Within plants	211	197·1832	0·9345
Total	266	256·6902	

$F_{9,211} = 3{\cdot}45$ $P \approx 0{\cdot}1$ per cent. $F_{211,46} = 1{\cdot}225$ $P > 20$ per cent.

Estimated environmental component of variance	0·8026
Approximately 95 per cent. fiducial limits	0·7906-0·8146
Estimated component of variance due to differences between progenies .	0·08497
Approximately 95 per cent. fiducial limits	0·02668-0·3040

It was not possible to partition the χ^2 further in order to investigate the differences between plants within progenies, as the expectations would be too small.

The analyses of variance for total growth and number of days to dehiscence are given in tables 5 and 6.

The tests of significance and estimates from the analysis of variance are inefficient because of the coarse grouping of the data, the scoring system used being effectively the grouping of a continuous distribution.

Thus the significance levels obtained from the tables, testing variation between progenies, are considerably different from those obtained from the contingency χ^2 and are less reliable. The differences between plants within progenies are not significant. This is to be expected as the progenies are the result of at least two generations selfing, and so the major part of the heritable variation should lie in the differences between the parents.

Since there was no significant difference between the S.S. within plants and the S.S. between plants within progenies, these were amalgamated and estimated components of variance calculated, using Sheppherd's correction (Kempthorne, 1950). The approximate fiducial intervals for the between-progenies components were calculated by the method of Bross (1950).

The correlation coefficient between the number of days to dehiscence and the score for total growth, is highly significant at 0·6136 for 266 d.f. This may mean either that there was a tendency to start recording flowers with a high initial score at an earlier stage in their growth, or that the two characters are in some way developmentally connected, or possibly both.

TABLE 6

Analysis of variance of number of days to dehiscence

	d.f.	S.S.	M.S.
Between progenies	9	29·4588	3·2732
Between plants within progenies .	46	52·1477	1·1336
Within plants	211	304·0855	1·4412
Total	266	385·6920	

$F_{9,211} = 2{\cdot}271$ P ≈ 2·5 per cent. $F_{211,46} = 1{\cdot}271$ P>20 per cent.

Estimated environmental component of variance	1·3028
Approximately 95 per cent. fiducial limits	1·2764-1·3292
Estimated component of variance due to differences between progenies .	0·0711
Approximately 95 per cent. fiducial limits	0·0097-0·2820

It may be noted that in a quantitative experiment of this kind, where measurements of a character are made on the flowers, the S.S. within plants gives a direct estimate of the environmental component of variance, without any assumptions concerning pure lines.

4. DISCUSSION

There are doubtless other factors, such as differential pollen-tube growth, which may affect the cross-fertilisation of the homostyle plants. However, a preliminary investigation did not show up any such differences. Most of the 1957 experiment took place in hot sunny weather, and so the results could not be explained by the sort of weather conditions that are known to favour late dehiscence.

Hence it seems that in considering the dynamics of the changes in the Primula populations containing homostyle plants, the cross-fertilisation of the latter must be taken into account.

The data given in the previous section indicate that, although there is evidence for polygenic control of the amount of cross-fertilisation, there is considerable environmental variation. Thus the amount of cross-fertilisation may be expected to vary from year to year, as is borne out by the results of the breeding experiment for 1955.

There exist three possibilities with respect to the tendency of the homostyles to cross.

(*a*) It is the residual of a tendency that existed in conjunction with the complete illegitimacy system.

(*b*) It is a tendency which has been selected for as the proportion of homostyles in populations increased, because of the advantage of outcrossing.

(*c*) The tendency existed with the complete illegitimacy system and has been further selected for as the proportion of homostyles increases.

By observing the number of days to dehiscence in pins from populations with a small proportion of homostyles, and studying the variation in the amount of crossing between homostyles from populations with different proportions of homostyles, it is hoped to gain some information about these possibilities. If (*a*) were true, the pins would also dehisce late and there would be no significant variation in the amount of crossing between homostyles from different populations. On the other hand, if (*b*) held, the pins would dehisce early, and the amount of crossing could be expected to vary more or less directly with the proportion of homostyles in a population, (*c*) being the intermediate possibility. If such a tendency to cross does exist with the illegitimacy system, it does not seem improbable that it arose out of selection favouring an increased efficiency of the illegitimacy system, a type of situation suggested by Mather and De Winton (1941). Whichever possibility is true, it does not appear that the change to homostyly in the populations is an abrupt change from an outbreeding to an inbreeding system (Crosby, 1949 and 1954).

Certainly the situation in the Primula populations is more complicated than has hitherto been supposed and it is hoped, soon, to publish a more detailed theoretical investigation of the possible explanations for the observed changes.

5. SUMMARY

In *Primula vulgaris*, a significant deficiency of pins was observed amongst the progeny of segregating homostyles, which had pollinated in open-pollination plots. An experiment on the homostyle flowers showed that their anthers dehisced late, and revealed a characteristic

pattern of relative growth of the stigma and anthers. These observations afford considerable evidence for the occurrence of natural crossing between homostyle plants of *Primula vulgaris*. This should therefore be taken into account when considering the dynamics of changes in the Primula populations and is probably a factor of some evolutionary significance.

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THE SEPARATION OF EPISTATIC FROM ADDITIVE AND DOMINANCE VARIATION IN GENERATION MEANS

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1. INTRODUCTION

MATHER (1949) introduced tests of generation means for epistasis which were elaborated by Cavalli (1952), Anderson and Kempthorne (1954), Jinks (1956) and Hayman (1957). Models of certain epistatic systems were described by Griffing (1950), Powers (1951) and Horner, Comstock and Robinson (1955). More general models were developed by Anderson and Kempthorne (1954), Hayman (1954*a*) and Hayman and Mather (1955) to describe the genetic variation present in two inbred lines and their descendant families. Anderson and Kempthorne showed in particular that all the information about additive, dominance and digenic epistatic variation available in the means of generations descended from two inbred lines is contained in just six parameters.

Here we describe the estimation from various experiments of six related parameters. We discover whether any useful measures of epistasis exist and investigate the problem of separating additive and dominance effects from epistatic effects.

2. THEORY

If two inbred lines differ by any number of unlinked genes the expectations of their, and some of their descendant, family and generation means may be expressed as

$$\begin{aligned}
P &= m + d - \tfrac{1}{2}h + i - j + \tfrac{1}{4}l \\
P' &= m - d - \tfrac{1}{2}h + i + j + \tfrac{1}{4}l \\
\mathrm{F}_1 &= m + \tfrac{1}{2}h + \tfrac{1}{4}l \\
\mathrm{F}_2 &= m \\
B &= m + \tfrac{1}{2}d + \tfrac{1}{4}i \\
B' &= m - \tfrac{1}{2}d + \tfrac{1}{4}i \\
\mathrm{F}_3 &= m - \tfrac{1}{4}h + \tfrac{1}{16}l \\
BS &= m + \tfrac{1}{2}d - \tfrac{1}{4}h + \tfrac{1}{4}i - \tfrac{1}{4}j + \tfrac{1}{16}l \\
BS' &= m - \tfrac{1}{2}d - \tfrac{1}{4}h + \tfrac{1}{4}i + \tfrac{1}{4}j + \tfrac{1}{16}l \\
\mathrm{F}_4 &= m - \tfrac{3}{8}h + \tfrac{9}{64}l \\
\textit{i.e.} \quad \text{mean} &= m + \alpha d + \beta h + \alpha^2 i + 2\alpha\beta j + \beta^2 l \qquad (1)
\end{aligned}$$

P and P' are the means of the two parent families and F_1 is the mean of their progeny. F_2, F_3 and F_4 are the means of generations descending from this cross by selfing. B and B' are the means of the first backcrosses to the parents while BS and BS' are the means of the progeny of selfing these first backcross families.

The genetic parameters d, h, i, j and l are sums of the gene parameters of Hayman and Mather (1955) as follows :—

$$d = \sum_{a} d_a\theta_a$$
$$h = \sum_{a} h_a$$
$$i = \sum_{a<b} i_{ab}\theta_a\theta_b$$
$$j = \tfrac{1}{2} \sum_{a \cdot b} (j_{ab}\theta_a + j_{ba}\theta_b)$$
$$l = \sum_{a<b} l_{ab}$$

m is the F_2 mean. Summation is over all genes a, b, ... by which the two inbred lines differ. The individual additive gene effects d_a, d_b, ... are taken to be positive and the state of association of the genes in the parents is indicated by the parameters θ_a, θ_b, ... which are positive unity when P contains the positive homozygote of the corresponding gene and negative unity when P' contains the positive homozygote. Thus d measures pooled additive effects and h pooled dominance effects while the epistatic i measures pooled interactions between additive effects, j between additive and dominance effects and l between dominance effects, all with due allowance for association. A parameter similar to h has already been used by Hayman (1954*b*) in connection with diallel crosses.

Anderson and Kempthorne's (1954) six parameters are related to ours by

$$K = m$$
$$E = \tfrac{1}{2}h$$
$$F = d - \tfrac{1}{2}h$$
$$G = \tfrac{1}{4}l$$
$$L = j - \tfrac{1}{2}l$$
$$M = i - j + \tfrac{1}{4}l.$$

Their E and G measure dominance and interactions between dominance effects, like our h and l, but their F, combining additivity and dominance, and their L and M, measuring pooled interactions of the constituents of F, are not so easy to interpret.

Another difference between these two sets of parameters should be explained. Those of Anderson and Kempthorne (1954) explicitly contain interactions between genes by which the parents differ and genes which are identical in the parents, whereas we have not mentioned these interactions. When, as in our case, one cross and its descendants are under consideration such epistasis may be incorporated in m, d and h and ignored as long as attention is confined to genetic material derived from the two parents. Of course, when several crosses with some parents in common are considered, such epistasis must be accounted for in the model because genes for which one pair of parents are identical may have alleles in other parents.

The parameters are fitted by the method of least weighted squares as outlined by Cavalli (1952). The necessity for different weights has a variety of causes. Most important is the reduction in the expected error of the means of later generations occasioned by the great numbers of individuals in these generations. As well as this statistical effect is the *generation effect* noted by Hayman (1957) in maize and cotton. This could be due to differences between heterozygous and homozygous generations or between segregating and genetically homogeneous generations. Stability may also be under genetic control (Jinks and Mather, 1955) and so vary from family to family. Thus each mean has its own error variance E_P, $E_{P'}$, E_{F_1}, etc., obtained from duplicates in the experiment. For example, if V_P is the variance between the n duplicate plots of one parent then $E_P = V_P/n$. We assume that n is large enough for these variances to be assumed reasonably constant. Further, no correlations between means are supposed to arise from the experimental layout.

The first step in the examination of the means is to fit m, d and h and to test for goodness of fit. If the χ^2 is significant m, d, h, i, j and l are fitted and tested. Let the estimates in the first step be $\hat{m}^*$, $\hat{d}^*$ and $\hat{h}^*$ and those in the second step $\hat{m}$, $\hat{d}$, $\hat{h}$, $\hat{\imath}$, $\hat{\jmath}$ and $\hat{l}$.

When epistasis is absent $\hat{m}^*$, $\hat{d}^*$ and $\hat{h}^*$ measure a constant, additivity and dominance. When epistasis is present and the data fit the six-parameter model $\hat{m}$, $\hat{d}$, $\hat{h}$, $\hat{\imath}$, $\hat{\jmath}$ and $\hat{l}$ measure a constant, additivity, dominance and the three kinds of epistasis. In these circumstances the deviations of the observed generation means from their expectations on the three-parameter model are epistatic in nature and reveal just what kinds of epistasis influence any given mean.

When epistasis occurs it would be convenient to be able to derive *epistasis-free* expectations of the generation means or, in other words, to discover what these means would have been if the genes had not interacted interallelically. We use expectations based on $\hat{m}^*$, $\hat{d}^*$ and $\hat{h}^*$, although these contain an epistatic element since m, d and h are not orthogonal to i, j and l in the equations of expectation. A better approach might seem to be to use the estimates of m, d and h from the six-parameter model to supply the expectations. Unfortunately such expectations are also not free of epistasis and we must delve in to the definitions of m, d and h in the presence of epistasis to understand why this should be so. Readers interested in the more practical side of this work should pass straight on to the experimental results.

The difficulty lies in a fundamental difference between genetic and statistical experiments. In a two-factor experiment, such as a nitrogen and phosphorus fertiliser trial with each fertiliser at three levels, the yield at one level of, say, nitrogen is obtained averaged over the three levels of phosphorus. If the fertilisers interact we still measure the mean yield at one level of nitrogen in the same way and we measure the interaction between any particular combination of

nitrogen and phosphorus by the difference between the corresponding observed yield and the average of the mean yields at the particular levels of nitrogen and phosphorus. As far as this experiment is concerned, unique measures of the effects of each fertiliser separately can be obtained whether the fertilisers interact or not. If now the experiment is repeated with the nitrogen at the same three levels but with the phosphorus at three different levels the yield at each level of nitrogen can still be obtained but, if the fertilisers interact, these yields may bear no relation to the yields at each nitrogen level in the first experiment. In other words, the effects of one factor cannot be compared between the two experiments because the other factor has altered. Of course, this often does not matter because each experiment may be sufficient in itself for the time and place at which it is performed.

The analogous genetical factors are genes, each with three levels, *AA*, *Aa* and *aa*. In a two-gene system the additive effect d_a of gene *A* may be measured by either $\frac{1}{2}(AABB-aaBB)$, $\frac{1}{2}(AABb-aaBb)$ or $\frac{1}{2}(AAbb-aabb)$ or by any average of these three quantities, and dominance h_a of gene *A* is measured by $AaBB-\frac{1}{2}(AABB+aaBB)$, $AaBb-\frac{1}{2}(AABb+aaBb)$ or $Aabb-\frac{1}{2}(AAbb+aabb)$ or by any average of these quantities. In a particular population the quantities would be averaged in proportion to the frequencies of the levels of the genetic factor *B*. In an F_2 family, for example, d_a would be an average of the three corresponding quantities above with frequencies $\frac{1}{4}$, $\frac{1}{2}$ and $\frac{1}{4}$ respectively. This one population is the analogue of one statistical experiment above. In an F_3 population the proportions would be $\frac{3}{8}$, $\frac{1}{4}$ and $\frac{3}{8}$ while for some purposes proportions $\frac{1}{2}$, 0 and $\frac{1}{2}$ are convenient. Each of these populations corresponds in our analogy to a single statistical experiment although the differences between our populations are ones of weight, and not of level, of the factors. If the two genes do not interact all these definitions of d_a and h_a are equivalent and d_a and h_a are defined uniquely but when digenic epistasis occurs the definitions of d_a and h_a in each population are different. Here lies the crux of the matter. One of our genetical experiments contains several populations, each analogous to one of a set of not easily comparable statistical experiments. Further, since we do not make comparisons within our populations, all our comparisons *must* be between populations in each of which our parameters should have different definitions.

Actually the situation is not as hopeless as it might seem, for the fact that the differences between our populations are differences in the proportions and not in the levels of the genetical factors enables us to relate the parameters in the various populations. As a standard it is convenient to define d_a, h_a, i_{ab}, j_{ab} and l_{ab} against an F_2 *background population* at the other loci controlling the character in question. These are the parameters we have introduced above and, with the exception of the constant term, are the parameters of Hayman and

Mather (1955). If $\bar{d}_a$, h_a, $\bar{i}_{ab}$, $\bar{j}_{ab}$ and l_{ab} are defined against a background population with proportions (p, q, r) at the other loci then Hayman (1954*a*) has shown that

$$\bar{m} = m + \sum_a [(p_a - r_a)d_a + (q_a - \tfrac{1}{2})h_a] + \sum_{a<b} [(p_a - r_a)(p_b - r_b)i_{ab} + (p_a - r_a)(q_b - \tfrac{1}{2})j_{ab} + (p_b - r_b)(q_a - \tfrac{1}{2})j_{ba} + (q_a - \tfrac{1}{2})(q_b - \tfrac{1}{2})l_{ab}]$$

$$\bar{d}_a = d_a + \sum_b [(p_b - r_b)i_{ab} + (q_b - \tfrac{1}{2})j_{ab}]$$

$$h_a = h_a + \sum_b [(p_b - r_b)j_{ba} + (q_b - \tfrac{1}{2})l_{ab}]$$

$$\bar{i}_{ab} = i_{ab}$$

$$\bar{j}_{ab} = j_{ab}$$

$$l_{ab} = l_{ab}$$

Here we have assumed that digenic, but no higher order epistasis, may occur. The inverse transformation is obtained by interchanging barred and unbarred parameters and changing the signs of $p - r$ and $q - \frac{1}{2}$. Evidently measures of digenic epistasis can be defined uniquely if higher order epistasis is absent but measures of additivity and dominance defined in one population must be corrected by epistatic terms for use in other populations.

If (p, q, r) describes the proportions in one of the generations in our list above then it may be shown from the above transformation that expectations in terms of parameters defined against a (p, q, r) background population may be written down as follows. Write m alone for the expectation of the mean of the generation in question. Write m together with appropriate terms in d and h for the other generations. Add i, j and l terms in accordance with equation (1).

A useful form is with $(p, q, r) = (\frac{1}{2}, 0, \frac{1}{2})$. Then

$$\bar{m} = m - \tfrac{1}{2}h + \tfrac{1}{4}l$$
$$\bar{d} = d - j$$
$$\bar{h} = h - l$$
$$\bar{i} = i, \bar{j} = j \text{ and } \bar{l} = l.$$

The expectations of the generation means become

$$P = \bar{m} + \bar{d} + \bar{i}$$
$$P' = \bar{m} - \bar{d} + \bar{i}$$
$$F_1 = \bar{m} + \bar{h} + \bar{l}$$
$$F_2 = \bar{m} + \tfrac{1}{2}\bar{h} + \tfrac{1}{4}\bar{l}, \text{ etc.,}$$

again in accordance with equation (1). This form has the important property that when a generation is selfed the mean of the resulting generation has an expectation derived from its parent's expectation by changing β to $\frac{1}{2}\beta$.

Reverting to our analogy between genetical and statistical experiments we see that we can overcome to some extent the lack of comparability of statistical experiments in our analogous genetical populations and can relate parameters defined in different populations.

Further, our measures of epistasis are actually identical in each population and when we estimate i, j and l from the generation means it does not matter which form the expectations take. However, the estimates of the constant and main effects, m, d and h, vary with the form of the expectations just as the estimates of the effect of each level of nitrogen varied between the two fertiliser trials. It is not possible, therefore, to obtain epistasis-free expectations of the generation means from m, d and h of the six-parameter model.

Here is another way of looking at the matter. In the absence of epistasis $2F_1-2F_2$ is a measure of dominance. With digenic epistasis and our standard form of the expectations

$$2F_1-2F_2 = h+\tfrac{1}{2}l.$$

In the F_1 form with $(p, q, r) = (0, 1, 0)$

$$2F_1-2F_2 = \bar{h}-\tfrac{1}{2}\bar{l}$$

while in the form with $(p, q, r) = (\tfrac{1}{2}, 0, \tfrac{1}{2})$ (and different $\bar{h}$ of course)

$$2F_1-2F_2 = \bar{h}+\tfrac{3}{2}\bar{l}.$$

Clearly, if these three forms are corrected for epistasis with the same value of l (or $\bar{l}$), different estimates of dominance must result.

Our approximation to epistasis-free expectations will, therefore, be derived from m, d and h estimated on the assumption of no epistasis —what we have labelled $\hat{m}^*$, $\hat{d}^*$ and $\hat{h}^*$. These expectations are independent of the definition of m, d and h and are unique.

Before considering experimental results it is useful to exhibit this theoretical discussion in matrix form. The equations of expectation become

$$\mathbf{y} = \mathbf{C}\mathbf{x}$$

which may also be written

$$\mathbf{y} = \mathbf{C}_1\mathbf{x}_1+\mathbf{C}_2\mathbf{x}_2$$

$\mathbf{y}$ is the vector of observed generation means. $\mathbf{C}_1$ is the matrix of coefficients of m, d and h and $\mathbf{C}_2$ is the matrix of coefficients of the epistatic i, j and l. $\mathbf{x}_1$ is the vector of the parameters m, d and h and $\mathbf{x}_2$ is the vector of i, j and l. If $\mathbf{E}$ is the (diagonal) matrix of error variances of the means then the estimates of the parameters are given by

$$\begin{pmatrix}\hat{\mathbf{x}}_1\\ \hat{\mathbf{x}}_2\end{pmatrix} = \hat{\mathbf{x}} = (\mathbf{C}'\mathbf{E}^{-1}\mathbf{C})^{-1}\mathbf{C}'\mathbf{E}^{-1}\mathbf{y} \text{ (where } \mathbf{C}' \text{ is the transpose of } \mathbf{C})$$

and the test of goodness of fit is

$$\chi^2 = \mathbf{y}'\mathbf{E}^{-1}\mathbf{y}-\mathbf{y}'\mathbf{E}^{-1}\mathbf{C}\hat{\mathbf{x}}$$

The error variances and covariances of the estimators are given by the matrix

$$\mathbf{V}(\hat{\mathbf{x}}) = (\mathbf{C}'\mathbf{E}^{-1}\mathbf{C}')^{-1}$$

After transformation to a different background population

$$\mathbf{x}_1 = \mathbf{A}\bar{\mathbf{x}}_1 + \mathbf{B}\bar{\mathbf{x}}_2$$

and

$$\mathbf{x}_2 = \bar{\mathbf{x}}_2$$

$\bar{\mathbf{x}}_1$ and $\bar{\mathbf{x}}_2$ have estimators

$$\hat{\bar{\mathbf{x}}}_1 = \mathbf{A}^{-1}\hat{\mathbf{x}}_1 - \mathbf{A}^{-1}\mathbf{B}\hat{\mathbf{x}}_2$$
$$\hat{\bar{\mathbf{x}}}_2 = \hat{\mathbf{x}}_2$$

so that the estimates of i, j and l are unaffected.

If epistasis is ignored the estimate of $\mathbf{x}_1$ is

$$\hat{\mathbf{x}}_1{}^* = (\mathbf{C}'_1\mathbf{E}^{-1}\mathbf{C}_1)^{-1}\mathbf{C}'_1\mathbf{E}^{-1}\mathbf{y}$$

and the expectations on this three-parameter model are

$$\hat{\mathbf{y}}^* = \mathbf{C}_1\hat{\mathbf{x}}_1{}^*$$

The test of goodness of fit is

$$\chi^2 = \mathbf{y}'\mathbf{E}^{-1}\mathbf{y} - \mathbf{y}'\mathbf{E}^{-1}\mathbf{C}_1\hat{\mathbf{x}}_1{}^*$$

and the variance matrix of the estimators is

$$\mathbf{V}(\hat{\mathbf{x}}_1{}^*) = (\mathbf{C}'_1\mathbf{E}^{-1}\mathbf{C}_1)^{-1}.$$

The expectation of the difference between observation and expectation on this three-parameter model is

$$\mathcal{E}(\mathbf{y} - \hat{\mathbf{y}}^*) = \mathbf{I}_1\mathbf{C}_2\mathbf{x}_2$$

where

$$\mathbf{I}_1 = \mathbf{I} - \mathbf{C}_1\mathbf{V}(\hat{\mathbf{x}}_1{}^*)\mathbf{C}'_1\mathbf{E}^{-1}.$$

Evidently these differences are due entirely to epistasis and their expected variances are given by

$$\mathbf{V}(\mathbf{y} - \hat{\mathbf{y}}^*) = \mathbf{I}_1\mathbf{E}.$$

3. RESULTS

At least six families or generations are necessary for the estimation of the six parameters m, d, h, i, j and l. The most convenient experiment involves P, P', F_1, F_2, B and B' : we give examples of tomato and *Nicotiana rustica* experiments of this kind. Another common type is the selfing experiment containing P, P', F_1, F_2, F_3, etc., which supplies estimates of m, i, h and l but fails to separate d from j : the latter occur only as d-j in P and P'. We give an example carried to F_4 in *Nicotiana rustica*. Finally we describe tomato and wheat experiments involving the first six means, together with BS and BS' in the tomato experiment, or F_3 in the wheat experiment.

(i) *Experiment* 1: P, P′, F_1, F_2, B *and* B′

Powers (1951) describes such an experiment on number of locules per fruit in the two tomato varieties Danmark and Johannisfeuer. He finds that on the logarithmic scale the data satisfy the essential

criterion of normality of the distributions in non-segregating generations. His results on this scale for two years are in the top of table 1A.

The 1939 data fail to fit a non-epistatic model ($\chi^2_3 = 11{\cdot}93$, $P = 0{\cdot}01 - 0{\cdot}001$) so that epistatic parameters should be fitted. The estimates of the parameters are in the lower half of the table, the left set in each case ignoring epistasis and the right (where relevant) allowing for it. Interactions between dominance effects (l) contribute the major portion of the epistasis: this agrees with Powers' (1951, p. 22) conclusion based on a two-gene model. Alongside the observations in the upper half of the table are the differences between them and their expectations on the three-parameter model. The epistasis suppresses the negative dominance in F_1 and enhances it in the F_2 and backcross generations as would duplicate epistasis.

TABLE 1A

Powers' (1951) Danmark × Johannisfeuer tomato cross

Mean number of locules per fruit (logarithmic scale)

	1939		1940
	Observation	Difference	Observation
P	$0{\cdot}7864 \pm 0{\cdot}0048$	$-0{\cdot}0033 \pm 0{\cdot}0015$	$0{\cdot}7739 \pm 0{\cdot}0080$
P'	$0{\cdot}9521 \pm 0{\cdot}0041$	$0{\cdot}0021 \pm 0{\cdot}0010$	$0{\cdot}9425 \pm 0{\cdot}0052$
F_1	$0{\cdot}7764 \pm 0{\cdot}0071$	$0{\cdot}0138 \pm 0{\cdot}0042$	$0{\cdot}7588 \pm 0{\cdot}0096$
F_2	$0{\cdot}8117 \pm 0{\cdot}0083$	$-0{\cdot}0029 \pm 0{\cdot}0078$	$0{\cdot}8119 \pm 0{\cdot}0133$
B	$0{\cdot}7595 \pm 0{\cdot}0071$	$-0{\cdot}0134 \pm 0{\cdot}0062$	$0{\cdot}7485 \pm 0{\cdot}0073$
B'	$0{\cdot}8404 \pm 0{\cdot}0083$	$-0{\cdot}0160 \pm 0{\cdot}0076$	$0{\cdot}8285 \pm 0{\cdot}0126$
	3-parameter model	6-parameter model	3-parameter model
m	$0{\cdot}8146 \pm 0{\cdot}0029$	$0{\cdot}8117 \pm 0{\cdot}0083$	$0{\cdot}8017 \pm 0{\cdot}0039$
d	$-0{\cdot}0835 \pm 0{\cdot}0030$	$-0{\cdot}0809 \pm 0{\cdot}0109$	$-0{\cdot}0864 \pm 0{\cdot}0044$
h	$-0{\cdot}1039 \pm 0{\cdot}0070$	$-0{\cdot}1399 \pm 0{\cdot}0505$	$-0{\cdot}1070 \pm 0{\cdot}0099$
i	.	$-0{\cdot}0470 \pm 0{\cdot}0397$	.
j	.	$0{\cdot}0020 \pm 0{\cdot}0113$	.
l	.	$0{\cdot}1387 \pm 0{\cdot}0569$	.
χ^2_3	11·93		5·85

difference = observed mean − expectation on three-parameter model.

The 1940 data fit a non-epistatic model. However, if estimates of m, d and h together with i, j and l are computed they are closely correlated to those of the previous year and indeed l just reaches significance again. This suggests that the lack of evidence for epistasis in the second year is due to a rise in the error variance rather than to a change in the action of the genes.

Jinks (1956) describes a diallel experiment on eight lines of *Nicotiana rustica* containing parental, F_1, F_2 and backcross generations. Hayman (1957) used the parental, F_1 and F_2 generations of this experiment to classify the individual crosses according to the type of

dominance and epistasis exhibited. We re-analyse the 1952 height data from three of the heterotic crosses to see the effect of the extra backcross information. The means, averaged over blocks and reciprocal crosses, are in table 1B. Family error variances are constant within generations over all crosses in the diallel experiment so that only one set of average standard errors is listed. Two of the crosses do have a parent in common but we ignore this fact because we are only using these experiments for illustration.

TABLE 1B

Jinks' (*1956*) Nicotiana rustica *crosses*

Mean height in inches

	1×2		3×4		4×5	General difference
	Observation	Difference	Obs.	Diff.	Obs.	
P	33·50±2·02	−1·41±1·06	27·40	−5·18	51·30	$0{\cdot}2210i-0{\cdot}2592j+0{\cdot}1467l$
P'	38·30±2·02	0·74±1·06	51·30	1·86	35·80	$0{\cdot}2201i+0{\cdot}2592j+0{\cdot}1467l$
F_1	51·00±1·43	−0·33±0·77	55·55	−1·66	53·20	$0{\cdot}2201i\quad\quad+0{\cdot}1467l$
F_2	39·58±1·19	−4·20±1·00	52·48	3·37	47·93	$-0{\cdot}2790i\quad\quad-0{\cdot}1033l$
B	49·45±1·71	6·33±1·45	48·85	3·96	51·80	$-0{\cdot}0290i+0{\cdot}3704j-0{\cdot}1033l$
B'	47·70±1·71	3·26±1·45	47·20	−6·13	46·40	$-0{\cdot}0290i-0{\cdot}3704j-0{\cdot}1033l$
	3-parameter model	6-parameter model	3-p. model	6-p. model	3-p. model	
m	43·78±0·65	39·58±1·19	49·11	52·48	48·45	Difference equals observed mean minus expectation on the 3-parameter model
d	−1·32±1·23	1·75±2·41	−8·43	1·65	7·14	
h	15·15±2·02	51·08±7·07	16·20	−1·62	9·65	
i	.	35·98±6·78	.	−17·82	.	
j	.	4·15±2·80	.	13·60	.	
l	.	−56·48±11·49	.	15·52	.	
χ^2_3	30·67		35·24		1·21	

The standard errors of each quantity are the same in every cross.

Since this P, P', F_1, F_2, B and B' experiment with error variances E_P, E_1, E_2 and E_B in the four generations is probably the most important of our experiments we give here the detailed expansion of the previous matrix formulas. When epistasis is absent, or is ignored, the estimates of m, d and h are

$$\begin{aligned}
2E^2\hat{m}^* &= 2E_2E_B(P+P'+2F_1)+(E_P+2E_1)(E_BF_2+E_2(B+B'))\\
(E_P+4E_B)\hat{d}^* &= 2E_B(P-P')+E_P(B-B')\\
E^2\hat{h}^* &= 2E_2E_B(2F_1-P-P')+E_1E_B(2F_2-P-P')+\\
&\quad 2E_1E_2(B+B'-P-P')+E_PE_B(F_1-F_2)+\\
&\quad E_PE_2(2F_1-B-B')
\end{aligned}$$

where $2E^2 = (E_P+2E_1)(2E_2+E_B)+8E_2E_B$

These may be tested for significance against

$$\begin{aligned}
2E^2 \operatorname{var}\hat{m}^* &= E_2E_B(E_P+2E_1)\\
(E_P+4E_B)\operatorname{var}\hat{d}^* &= 2E_PE_B\\
E^2 \operatorname{var}\hat{h}^* &= 4E_1E_2E_B+2E_PE_2E_B+2E_PE_1E_B+4E_PE_1E_2
\end{aligned}$$

The test for epistasis is a χ^2 with three degrees of freedom which may be written

$$\{E_B(P+P'+2F_1-4F_2)^2+2E_2(P+P'+2F_1-2B-2B')^2+$$
$$(E_P+2E_1)(2F_2-B-B')^2\}/4E^2+(P-P'-2B+2B')^2/(2E_P+8E_B)$$

The squared terms are linear combinations of the A, B and C used by Mather (1949) to test for epistasis.

When epistasis is present the six parameters provide an exact fit to the generation means and their estimates are

$$\begin{aligned}
\hat{m} &= & & & F_2 \\
\hat{d} &= & & & B- B' \\
\hat{h} &= & -\tfrac{1}{2}P-\tfrac{1}{2}P'+ & F_1-4F_2+2B+2B' \\
\hat{i} &= & & -4F_2+2B+2B' \\
\hat{j} &= & -\tfrac{1}{2}P+\tfrac{1}{2}P' & + B- B' \\
\hat{l} &= & P+ P'+2F_1+4F_2-4B-4B'
\end{aligned}$$

Since these equations do not involve the error variances they were also used with the previous data of Powers. The expected variances and covariances of the parameters are linear combinations of the error variances. For example,

$$\begin{aligned}
\text{var}\, j &= \tfrac{1}{2}E_P+2E_B \\
\text{cov}(i, l) &= -16E_2-16E_B
\end{aligned}$$

The first two *Nicotiana rustica* crosses in table 1B exhibit epistasis—i and l types in the first and i and j types in the second. All three show dominance, but not necessarily additive, variation—which is not surprising in experiments selected for heterosis. At the right of the table are the expectations in terms of i, j and l of the differences between observed and expected means. The relative importance of the three kinds of epistasis to these deviations can be seen at a glance.

We can compare these results with Hayman's (1957) results based on parental, F_1 and F_2 data alone. Hayman classified crosses as complementary epistatic, non-epistatic or duplicate epistatic: in a non-epistatic cross the F_2 mean lay midway between the midparent and the F_1 mean, in a complementary cross the F_2 mean was nearer to the midparent and in a duplicate cross nearer to the F_1 mean, the deviations of the F_2 mean from the midway point being proportional to $2i+l$ in our present notation. It is clear from table 1B that 1×2 would be complementary, 3×4 duplicate and 4×5 non-epistatic on this system of classification.

The addition of backcross information reveals a more complicated situation. Whereas in the tomato data in table 1A the backcross deviations followed the F_2 deviations, here the epistatic deviations in the backcrosses are generally opposite to those in the F_2 and there are also differences between the two backcrosses themselves. The simple one-way classification breaks down and a three-way classification in terms of i, j and l is necessary.

It also follows that the relationship between epistasis and heterosis cannot be as simply characterised as in Hayman's (1957) paper. Comparisons between the midparent deviation on the one hand and either the F_1, F_2 or mean backcross deviations on the other hand should reveal the effect of epistasis on apparent dominance, or more correctly on heterosis since all three crosses were selected as heterotic. Now for statistical reasons the difference between observation and expectation for the midparent happens to be the same as the difference for the F_1 in this experiment (see final column of table 1B) so that we must turn to the F_2 and mean backcross for information. The deviations in F_2 are in agreement with Hayman's (1957) classification, *viz.* in 1 ×2 epistasis depresses the F_2 relative to the midparent, in 3 ×4 it enhances it while in 4 ×5 it leaves it unaffected. The deviations in the mean backcross are just opposite in sign to the F_2 deviations in 1 ×2 and 3 ×4 and, of course, negligible in 4 ×5. The evidence from these three crosses supports Hayman's (1957) conclusion that, in *Nicotiana rustica*, heterosis may be enhanced or diminished by epistasis or may occur independently of it.

(ii) *Linkage*

Linkage only affects the epistatic terms in the generation means. While failure to fit the non-epistatic model (*m*, *d* and *h*) is a definite indication of epistasis, failure to fit the digenic epistatic model (*m*, *d*, *h*, *i*, *j* and *l*) may indicate either trigenic epistasis or linkage or both. If p_{ab} is the linkage between genes *a* and *b* the estimators of the parameters in the present experiment have expectations

$$\hat{m} = m$$
$$\hat{d} = d$$
$$\hat{h} = h - \sum_{a,b} (1-2p_{ab})(i_{ab}\theta_a\theta_b + j_{ab}\theta_a + j_{ba}\theta_b - 2p_{ab}l_{ab})$$
$$\hat{i} = i - \Sigma(1-2p_{ab})(i_{ab}\theta_a\theta_b + j_{ab}\theta_b + j_{ba}\theta_b - 2p_{ab}l_{ab})$$
$$\hat{j} = j$$
$$\hat{l} = l + \Sigma(1-2p_{ab})(2j_{ab}\theta_a + 2j_{ba}\theta_b - (1+2p_{ab})l_{ab})$$

Three of the parameters, *m*, *d* and *j* and the difference *h*-*i* are still estimated correctly but *h* and *i*, separately, and *l* are disturbed by epistatic linkage terms. It is not possible to determine the two extra terms which have appeared here by adding later generations because the powers of *p* are higher, and these two extra terms do not appear, in the expectations of later generation means (Hayman, 1954*a*).

(iii) *Experiment* 2: P, P′, F_1, F_2, F_3 *and* F_4

Three lines of *Nicotiana rustica* (which were also lines 1, 2 and 4 of Jinks' (1956) diallel experiment) were set out as a small diallel in which each cross was selfed up to the F_4 generation. The complete experiment was grown in Birmingham in 1954 and again in 1955.

Table 2 contains the mean heights and standard errors of each family or generation of the cross 1 ×2 and its descendants in the two years.

The expectations of the means may be written

$$P = m+d' -\tfrac{1}{2}h+i+\tfrac{1}{4}l$$
$$P' = m-d' -\tfrac{1}{2}h+i+\tfrac{1}{4}l$$
$$F_n = m-(\tfrac{1}{2}-2^{-n+1})h+(\tfrac{1}{2}-2^{-n+1})^2 l \qquad n = 1, 2, 3, 4$$

where $d' = d-j$. Only five parameters can be estimated from this

TABLE 2

Hayman's Nicotiana rustica *selfing experiment*

Mean height in inches

	1954		1955	
	Observation	Difference	Observation	Difference
P	41·42±0·49	0·31±0·14	32·78±0·34	−0·84±0·10
P'	39·30±1·09	1·54±0·69	42·02±0·78	−4·37±0·54
F_1	46·46±0·89	−0·45±0·28	52·18±0·60	−0·84±0·21
F_2	45·72±1·49	2·55±1·42	46·64±0·92	0·13±0·88
F_3	47·73±1·44	6·43±1·40	43·86±0·64	0·60±0·60
F_4	38·36±0·57	−2·00±0·43	43·53±0·35	1·89±0·26
	3-parameter model	5-parameter model	3-parameter model	5-parameter model
m	43·17±0·44	48·25±1·33	46·51±0·28	46·26±0·76
d'	1·68±0·53	1·06±0·60	−6·39±0·37	−4·62±0·43
h	7·48±1·02	12·78±1·50	13·02±0·69	9·36±0·95
i	.	6·81±1·42	.	−5·44±0·90
l	.	−33·27±7·70	.	5·07±4·55
χ^2_3	37·99		68·72	
χ^2_1		14·31		0·54

Difference = observed mean−expectation on the 3-parameter model.

experiment : four, m, h, i and l, are as before but d', measuring the spread of the parents, is a compound of additive effects and interaction between additive and dominance effects.

A χ^2 with three degrees of freedom tests for epistasis and a χ^2 with one degree of freedom for linkage or trigenic epistasis. In 1955 digenic epistasis is present and fig. 1 shows the relations between the various expectations. The full line joins the midparent and the other successive generation means. Heterosis is marked here. The broken line joins expectations derived from the best fitting simple m, d and h model. The dotted line joins expectations on the complete five-parameter model. The epistatic terms account well for the failure of the generation means to fall with inbreeding as they should on a simple dominance model. Epistasis is responsible for some of the heterosis because the excess of the F_1 over the midparent is significantly less for the three-parameter expectations than for the observed means.

In 1954 even the five-parameter model fails to account for the variation between the generation means. It appears that epistasis is suppressing heterosis in the F_1 but a more comprehensive model might reveal otherwise.

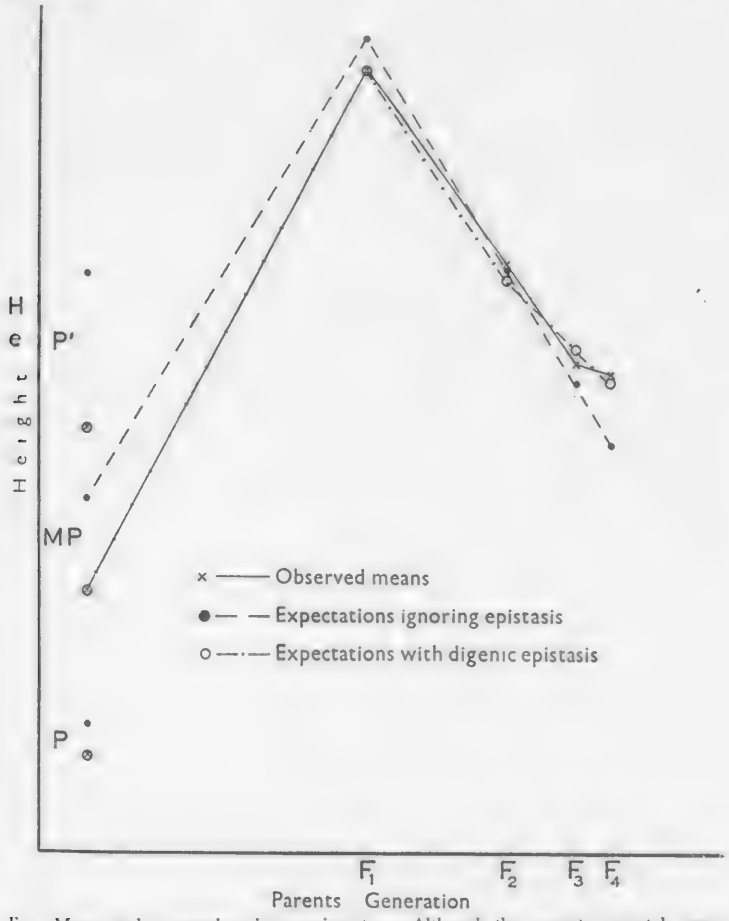

FIG.—Means and expectations in experiment 2. Although the separate parental means are indicated it is the midparent that is joined to the F_1, F_2, F_3 and F_4 means.

Before passing on to other experiments it is interesting to draw together the information about the incidence of epistasis in the *Nicotiana rustica* cross 1 ×2. In 1952 and 1953 this cross was grown in the form of our experiment 1 as part of a diallel experiment of Jinks (1956) while in 1954 and 1955 it was grown by ourselves in the form of our experiment 2 as part of a small diallel experiment. We have reported the results in all these years, except in 1953 when epistasis

is not significant. The incidence of epistasis varies greatly from year to year. In 1953 epistasis is absent—and not through a rise in the environmental variation in that year because genetic and environmental variation are both about 50 per cent. higher than in 1952. The parameters h, i and l are significant and similar in sign in 1952 and 1954 but i changes sign and l disappears in 1955. The only other parameter common to all years is $d' = d\text{-}j$ which is significantly

TABLE 3

Powers' (1955) Criolle×Sioux tomato cross

	Weight per locule (log scale)		Weight per fruit (log scale)	
	Observation	Difference	Observation	Difference
P	0·3116±0·0168	0·0194±0·0115	1·1558±0·0143	0·0203±0·0101
P'	1·2248±0·0286	0·0170±0·0231	2·1017±0·0250	0·0182±0·0210
F_1	0·8732±0·0222	0·0116±0·0148	1·5431±0·0210	0·0054±0·0150
F_2	0·8020±0·0189	−0·0038±0·0176	1·5775±0·0153	0·0039±0·0142
B	0·5544±0·0157	−0·0225±0·0134	1·3265±0·0119	−0·0101±0·0098
B'	1·0682±0·0167	0·0335±0·0141	1·8479±0·0161	0·0373±0·0142
BS	0·5479±0·0107	−0·0011±0·0082	1·3489±0·0082	−0·0057±0·0061
BS'	0·9835±0·0137	−0·0233±0·0100	1·8132±0·0098	−0·0154±0·0064
	3-parameter model	6-parameter model	3-parameter model	6-parameter model
m	0·8058±0·0068	0·7992±0·0087	1·5736±0·0058	1·5697±0·0071
d	−0·4578±0·0105	−0·4882±0·0196	−0·4740±0·0084	−0·5017±0·0164
h	0·1116±0·0241	0·1592±0·0409	−0·0718±0·0210	−0·0115±0·0333
i	.	0·0468±0·0430	.	0·0655±0·0351
j	.	−0·0454±0·0276	.	−0·0408±0·0238
l	.	−0·0212±0·0955	.	−0·0757±0·0839
χ^2_5	11·51		11·72	
χ^2_2		4·93		3·82

Difference = observed mean − expectation on the 3-parameter model.

negative in both 1952 and 1955 but negligible in 1954. The relation of epistasis to heterosis is similarly variable, epistasis increasing heterosis in 1952 and 1955, decreasing it in 1954 and leaving it unaffected in 1953. Without going into any more detailed argument it is clear that in height in this species epistasis is under the influence of the seasons. Hayman (1957) confirms this by finding a correlation of 0·02 between epistasis in 1952 and 1953 in a diallel of 28 crosses.

(iv) *Experiment* 3: P, P′, F_1, F_2, B, B′, BS *and* BS′

Powers (1955) investigated number of locules, weight per locule (W/L) and weight per fruit (W/F) in the cross between the tomato varieties Criolle and Sioux. The logarithms of W/L and W/F are normally distributed in non-segregating generations. Table 3 contains the means of these two characters in each family or generation.

The χ^2 with five degrees of freedom supplies some evidence (P =

0·05) for epistasis in both characters. None of the epistatic parameters i, j and l is actually significant in either character but some linear combinations of them must be; for example, those combinations of i, j and l which form the differences between observation and expectation on the three-parameter model in generations B', BS', and possibly P, are significant.

The similarity between the epistasis in the characters W/L and W/F has a simple explanation in terms of Powers' third character, number of locules (NL). If the non-normality of the parental and F_1 distributions of log(NL) is ignored the χ^2 with five degrees of freedom can be computed for this character, too. This reveals no evidence of epistasis. Now log(W/F) is the sum of log(W/L) and

TABLE 4

Copp's (unpublished) cross 7× Tainui wheat cross

Percentage shattering (angular scale)

	Observation	Difference		3-parameter model	6-parameter model
P	41·0±0·90	−2·71±0·56	m	40·09±0·31	41·26±0·39
P'	36·0±0·90	−3·30±0·56	d	2·20±0·58	0·90±1·34
F_1	34·3±1·05	−4·37±0·71	h	−2·83±1·08	−5·30±1·28
F_2	41·3±0·67	1·21±0·54	i	.	−1·11±1·13
B	41·4±0·95	0·21±0·85	j	.	−1·60±1·49
B'	40·5±0·95	1·52±0·85	l	.	−17·21±4·02
F_3	41·5±0·32	0·71±0·21			
			χ^2_4	50·19	
			χ^2_1		0·01

Difference = observed mean−expectation on the 3-parameter model.

log(NL) so that epistasis in log(W/F) should be similar in nature to epistasis in log(W/L). Dominance, on the other hand, takes different signs in log(W/L) and log(W/F), reflecting the presence of dominance in log(NL). These conclusions agree with Powers' (1955) gene model.

(v) *Experiment* 4: P, P', F_1, F_2, B, B' *and* F_3

The wheat cross Cross 7 ×Tainui and descendant generations was grown by Mr L. G. Copp in 1950-51 as part of an experiment at the Crop Research Division, Lincoln, New Zealand. Among the grain characters observed was percentage shattering. This character, measured on an angular scale, was normally distributed in parents and F_1. Table 4 contains the means and variances of each family; families of the same kind (the two parents or the two backcrosses) have similar variances which are pooled.

The upper half of the table shows that negative dominance amounting almost to heterosis is present in F_1 but this reverses to positive dominance in F_2, F_3 and the backcrosses. This is indicative of the l-type of epistasis which appears in the lower half of the table. The other types of epistasis are of no importance.

4. DISCUSSION

We have presented an investigation of the genetic variation in the family means of four experiments.

All these experiments can be tested for their fit to a simple additive and dominance genetic model. If this fit is bad three of the experiments (2, 3 and 4) can be tested for their fit to a digenic epistatic model in which the genes are not linked.

In the absence of epistasis all the experiments provide estimates of additivity and dominance. When epistasis is present experiments 1, 3 and 4 provide estimates of the three kinds of epistasis as well, but, as we have explained, the measures of additivity and dominance are no longer unique and are valid only in a particular genetic population. This means that the influence of epistasis on, for example, heterosis cannot be fully assessed although a comparison between observation and expectation on the three-parameter model gives some indication of this influence.

It would be a great advantage if an experiment could be designed in which the estimates of m, d and h were independent of epistasis. Quite apart from the difficulty in defining m, d and h in the presence of epistasis it is easy to see from equation (1) that such a design is not possible. In statistical terms the requirement is that m, d and h be orthogonal to i, j and l in the expectations of family means. Since the coefficients of m, i and l are all positive m cannot be orthogonal to i or l.

With the ideal experiment unattainable it becomes more important to assess the accuracy with which the present experiments estimate and separate the various parameters. So as to compare the experiments on a standard basis we shall suppose that all observed means have unit error variances. Table 5 contains variance-correlation matrices of the estimators of the parameters in each of the four experiments. The error variances lie on the diagonals and the error correlations off the diagonals of each matrix.

Experiment 1 is the simplest that permits estimation of all six parameters but the high error variances of $\hat{h}$, $\hat{\imath}$ and $\hat{l}$ show that much less information is available about these parameters than about m, d and j. Further, many of the estimators are highly correlated, especially the pair, $\hat{d}$ and $\hat{\jmath}$, and the trio, $\hat{h}$, $\hat{\imath}$ and $\hat{l}$. The addition of F_3 to the experiment proves to be more rewarding than the addition of BS and BS', especially in the information supplied about h and i. l remains inaccurately estimated in all the experiments. Amongst the correlations, that between $\hat{d}$ and $\hat{\jmath}$ remains high in all three experiments, but all the other correlations are considerably reduced in the larger experiments and especially in the F_3 experiment. In experiment 2, d and j are, of course, fully correlated and cannot be estimated separately but this is the best experiment for estimating h and i. It is also advantageous from a practical point of view with a species such as wheat where much labour is required to produce sufficient seed from a cross.

A more comprehensive measure of the information supplied by an experiment was proposed by Wilks (1932). Just as the reciprocal of the variance of a single variable is a measure of the information about that variable Wilks suggests the use in the multivariate case of the reciprocal of the pth root of the determinant of the variance-covariance matrix of the p variables. This would measure the mean information per variable and is in effect the reciprocal of the geometric mean

TABLE 5

Error variances (diagonal terms) and correlations (off diagonals) of the estimators of m, d, h, i, j *and* l *in the four experiments on the assumption of unit error variances of the observed means. I = mean information per parameter*

Experiment 1

	m	*d*	*h*	*i*	*j*	*l*
m	1·00	0·00	−0·79	−0·82	0·00	0·54
d		**2·00**	0·00	0·00	0·89	0·00
h			**25·50**	0·97	0·00	−0·84
i				**24·00**	0·00	−0·89
j					**2·50**	0·00
l	I = 0·5000					**54·00**

Experiment 2

	m	*d′*	*h*	*i*	*l*
m	0·84	0·00	0·52	0·51	−0·84
d′		**0·50**	0·00	0·00	0·00
h			**3·27**	0·77	−0·57
i				**3·11**	−0·78
l	I = 0·7019				**42·21**

Experiment 3

	m	*d*	*h*	*i*	*j*	*l*
m	0·31	0·00	−0·34	−0·44	0·00	0·03
d		**1·62**	0·00	0·00	0·89	0·00
h			**7·97**	0·91	0·00	−0·71
i				**8·17**	0·00	−0·82
j					**2·29**	0·00
l	I = 0·6734					**31·67**

Experiment 4

	m	*d*	*h*	*i*	*j*	*l*
m	0·30	0·00	−0·16	−0·25	0·00	−0·26
d		**2·00**	0·00	0·00	0·89	0·00
h			**4·66**	0·83	0·00	−0·53
i				**4·20**	0·00	−0·72
j					**2·50**	0·00
l	I = 0·6960					**22·55**

of the variances of the variables with due allowance for correlation between the variables. This mean information per variable is included as I in table 5. Experiment 3 supplies about 35 per cent. more information and experiment 4 about 39 per cent. more information than experiment 1. This is in line with our general discussion above and shows that the addition of the single F_3 generation to experiment 1 is on the whole more informative than the addition of the two selfed backcross generations.

One reason for presenting so many experimental results has been to show that epistasis occurs widely and that it may be as important as additivity or dominance in genetic variation. Anderson and Kempthorne (1954) also give an example of our experiment 2 in which epistasis is as important as dominance.

The second reason is to show the variety of epistatic forms. Our i, j and l, representing the influence of the three kinds of epistasis on means, may occur in any combination of sign and magnitude. In particular, experiment 1B shows that epistasis may enhance or diminish heterosis.

The third reason is to investigate the stability of epistatic effects under change of environment. The little evidence from tomato yield in experiment 1A suggests stability of gene action from year to year. Height in *Nicotiana rustica*, however, seems from a comparison of experiments 1B and 2 on the cross 1 ×2 to exhibit stability of dominance but instability of epistasis under seasonal variation. This was also Hayman's (1957) conclusion from 28 crosses grown in two years.

The experiments lead, therefore, to the expected result. Each species and each character has its own mode of action and interaction of the controlling genetic material.

The consequences of our theoretical discussions are not so simple. We have seen that epistasis is an important component of genetic variability and that sufficiently large experiments permit accurate estimation of mean epistatic effects by simple statistical procedures. Although opposing epistases will remain undetected the same is true of dominance and additivity so that our comparisons between the various modes of gene action are on an equitable basis. It should be more informative to measure mean square genetic effects but Hayman (1954*a*, 1955) has shown that the expectations of variances are complicated. Even in experiment 2 the expectations of variances in terms of digenic effects require eight components, and the inclusion of backcross generations would require even more components, unlike the situation with the means where the same six components suffice for all mating combinations descended from two inbred lines. Our measures of epistasis are probably some of the simplest and most reliable that may be devised. However, while we are able to detect and measure epistasis with some confidence, we have uncovered a problem in the proper description of the main effects (additivity and dominance). Earlier we approached this problem through a statistical analogy and now we shall propound a more genetical approach.

Suppose that the two parents P and P' are genetically identical. Then the expectations of all the family or generation means are the same, say m, and the weighted average of these means is the best estimate of the level of expression of the single genotype involved in the experiment. Even if the parents were genetically different this estimate of m would still be a useful norm from which to measure the differences in expression of the various genotypes and, further, this estimate would be unique whatever the mode of gene action. Now suppose that P and P' differ by genes that do not interact non-allelically. Then we may arbitrarily take m to be the expectation of any mean, such as F_2 in our case, or even of a hypothetical mean, such as the midparent, and add appropriate terms in d and h to complete the expectations of the other means. The estimates of d and h are unique but the estimate of m depends on the form of the expectations. Indeed, m no longer represents a norm of gene action but merely a particular (background) population such as F_2, midparent, etc. d and h, while nominally defined on the basis of the same

background population are independent of it because of the absence of epistasis. The expectations of the means derived from these m, d and h are also unique in spite of the arbitrary nature of m. Further, even if epistasis did occur amongst the genes by which P and P' differ, the uniqueness of the estimates, ignoring epistasis, of d and h and the corresponding expectations would still be preserved: in this case the expectations would represent the nearest possible non-epistatic system and d and h the main effects in that system. These are the expectations from which we derive the differences in tables 1-4.

The next step is to suppose that P and P' differ by genes exhibiting digenic, but no more complex, epistasis. Then the model of this paper applies: i, j and l are unique measures of epistasis with unique estimates but all three of m, d and h depend, in definition directly, and in their estimates indirectly through the form of the expectations, on an arbitrary background population. The expectations derived from the estimates of the six parameters are unique but m, d and h and expectations derived from their estimates have little use. If we proceed to the fourth stage and admit trigenic epistasis the argument moves up a further step. We add a final simple example from epistacy between two major genes. Here it is not possible to use the description "A is dominant to a" without a qualification such as "only in the presence of the dominant allele B".

The implication of this general discussion is that when epistasis occurs main effects become somewhat intangible. As we have explained here, and as Hayman (1955) has also explained in a slightly different context, the reason lies in our use of ordinary statistical constructs in the genetical situation with its relationships between individuals, laws of segregation, etc., that have no counterpart in ordinary statistical experiments. The complete separation of main effects from epistasis does not seem to be feasible and even a description of gene action jointly in terms of main effects and epistasis seems to be possible only where each gene and each possible genotype in the genetic system can be identified. Our method of considering the deviations of observation from the best-fitting non-epistatic model in terms of the epistasis actually present does, however, give some idea of the relationship between epistasis and additivity and dominance.

5. SUMMARY

Five experiments consisting of various descendants from two inbred lines from various species are described. The means of families or generations are influenced by epistasis, often to as great an extent as by additive or by dominance variation. This epistasis may be in the form of interaction between additive effects, between dominance effects or between additive and dominance effects, and all forms occur in various combinations and with varying sign.

The simplest experiment supplying information on additive, dominance and the three kinds of epistatic variation contains two

inbred lines and their F_1, F_2 and first backcross generations. Not all the genetic parameters are estimated with equal accuracy or without correlation and some improvement in accuracy and independence is gained by adding selfed backcross generations or even more by adding an F_3 generation.

The measurement of additivity and dominance in the presence of epistasis is found to pose a problem. A measure of, say, dominance at a locus depends on the genetic state of other loci interacting with it, so that a unique measure of dominance can only be constructed by arbitrary specification of the state of the other loci. The difficulty can be avoided, but not entirely overcome, by finding the best-fitting (unique) non-epistatic model and considering its deviation from observation in terms of the epistasis present.

Acknowledgments.—Thanks are due to Dr J. L. Jinks of Birmingham, England, and the Agricultural Research Council of Great Britain for permission to use the data in table 1B, to the Agricultural Research Council of Great Britain for the data in table 2, to Mr L. G. Copp of Lincoln, New Zealand, and the Department of Scientific and Industrial Research, New Zealand, for the data in table 4, and to Miss E. Stevenson of this Laboratory for assistance with the computations.

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REVIEWS

VARIATION AND HEREDITY. By H. Kalmus. London: Routledge and Kegan Paul. 1958. Pp. 227+xi. 28s.

This work is part of a survey of human biology and in it Dr Kalmus has discussed various aspects of human variation with particular reference to its causation. There are useful sections on the application of modern genetics to problems of clinical medicine, genetic counselling, racial intermarriage, eugenics and the genetic aspects of radiation hazards.

Human variation cannot be understood without a knowledge of simple Mendelian genetics. Consequently the author of a book such as this one should either provide an explanation of simple Mendelism or say that he is assuming that the reader has such elementary knowledge. Kalmus does provide sections on genetics, but, in my opinion, the text is too advanced for the beginner and too elementary to be useful to most students with a more advanced knowledge. It would have been better to have left out much of the text devoted to explaining genetics and to have used the space for a more extended treatment of the application of genetics to human affairs.

The text is fairly free of misprints and errors but unfortunately the same cannot be said of many of the figures. I have detected fifteen more or less serious errors in fig. 9 by comparing it with the original. Six blood-group determinations have been omitted from fig. 8 and four of these give important information on linkage. Furthermore, the reference to the original source is incorrect. The text of fig. 6 is inadequate since it suggests that the pedigree excludes close linkage between the P blood-group system and ectrodactyly, whereas neither the figure nor the data in the original paper quoted even exclude the possibility that the two are absolutely linked ! The reference in the text to fig. 5 is not given in the bibliography and the number 96·44426 in table 8 should, I think, be 96·44303.

Despite these errors the text can be recommended to students of human variation who wish to read an unbiased account of human variation in relation to heredity. In fact the author sometimes appears to go to considerable lengths in order not to reveal his own personal opinion on certain topics. The book should be particularly useful to those geneticists who are not accustomed to working with data on human genetics.

P. M. SHEPPARD.

UNCOMMON PEOPLE. A study of England's Elite. By Paul Bloomfield. London: Hamish Hamilton. 1956. Pp. 210+xi. 21s.

An account of the pedigrees and breeding systems of certain intellectually outstanding lines contributing to the British governing class since the sixteenth century: notably, the Villiers, Cecil, Wedgwood, Darwin, Strachey, Penrose, Barclay and Buxton Connections. The study of this evidence should be widely interesting to geneticists who will wish to follow up the references and quotations. Unfortunately there is no list of sources.

C. D. D.

BREEDING BETTER COWS. By Craig Wheaton-Smith. Ipswich: Dairy Farmer (Books) Ltd. 1957. Pp. 191. 16s.

Cattle breeding in advanced countries is now in a state of rapid transition as a result of the impact of genetics and artificial insemination on the traditional methods developed by the pedigree breed societies.

This book is the work of one of the most enterprising of British cattle breeders as well as one of the most successful. It explains in genetic terms the history and purpose of cattle breeding. It exposes the fallacies and errors of traditional methods and attempts to show how genetic and also physiological principles can now be applied to the breeding of dairy and beef cattle. It includes a remarkable bibliography, a glossary and much factual information. C. D. D.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND TWENTY-SIXTH MEETING of the Society, held on 2nd and 3rd APRIL 1958, at the UNIVERSITY OF READING

EXPERIMENTS ON THE INDUCTION OF MUTATIONS IN CROP PLANTS

G. J. DOWRICK and W. WILLIAMS
John Innes Horticultural Institution, Bayfordbury

Experimental work on the use of P^{32} as a mutagen on various species of crop plants is presented. The distribution of radio-activity in various parts of the plants has been measured and the biologic half-life of the isotope was found to be substantially less than its physical half-life owing to exudation through the roots.

Mutant phenotypes identical with those existing in wild populations were recovered. These were characterised by differences in growth rate as compared with both the wild type and the naturally occurring mutants. Evidence is presented which indicates that the variability of complex quantitative characters is increased in irradiated material, and that selection for maximum character expression is therefore made more effective.

THE USE OF ARTIFICIALLY INDUCED MUTATIONS IN CROP IMPROVEMENT

R. N. H. WHITEHOUSE
Plant Breeding Institute, Cambridge

Plant Breeders are facing a challenge from the claims made for mutation breeding. They must decide what should be their response to this new method. An attempt will be made to analyse the problems confronting the breeder who wishes to use mutagenic agents for crop improvement. The techniques involved will be considered with reference to self-pollinated, cross-pollinated and vegetatively propagated plants.

It is suggested that in the present state of our knowledge, mutation breeding is of very limited value but that there is need for much more fundamental work on the mutation process.

CYTOPLASMICALLY DETERMINED VARIATION IN *ASPERGILLUS NIDULANS*

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Strains of *A. nidulans* carrying nutritional markers and also resistant (single gene determined) to acriflavine undergo irreversible changes after serial subculture on medium with acriflavine. Controls without acriflavine are unchanged. Three such modified strains have so far been studied. They bear few or no conidia and are described as " mycelial ". They are stable on repeated subculture on any medium so far tested, with or without acriflavine. The mycelial condition is cytoplasmically determined and can be transmitted infectively to other genotypes (with or without

an allele for acriflavine resistance) by heterokaryosis. Nucleo-cytoplasmic interactions are pronounced and each mycelial cytoplasm gives a wide range of phenotypes in combination with different genotypes. The three original mycelial strains have been shown to be cytoplasmically different by combining each cytoplasm with a common genotype; the three strains so obtained were phenotypically quite distinct. Heterokaryotic combinations of pairs of mycelial cytoplasms do not restore the non-mycelial type.

Balanced heterokaryons (and heterozygous diploids derived from them) between a mycelial and a non-mycelial type are almost normal suggesting that the mycelial condition is little expressed in a prototroph. However, mitotic recombination in such diploids gives types of which a proportion are mycelial. Meiotic recombination in crosses between a mycelial and a non-mycelial gives both mycelial and non-mycelial types. Preliminary results suggest that segregation of the mycelial condition at meiosis is irregular. At mitotic recombination in heterozygous diploids the determination of a mycelial versus a non-mycelial phenotype appears to be closely correlated with the particular genotype produced at crossing-over or haploidisation. That is, certain genotypes appear always to express the mycelial phenotype while other genotypes express the non-mycelial phenotype.

UNEXPLAINED RESPONSE OF MICE TO SELECTION FOR LACTATION

N. BATEMAN

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Selection for lactation in mice has been carried in both upward and downward directions through 20 generations. A number of reverse selections, made in parallel with the two forward lines, helped to assess the magnitude of heritable variation within the lines. The divergence for each generation between the progenies of forward and reverse selected parents indicated that heritability was one-half and did not change during the experiment. Nevertheless, the rate of divergence between the two forward selected lines declined in successive generations so that, by the 20th, only one-sixth of the total forward selection applied was realised as a difference between lines. Thus, on average, a five-fold change in response followed a change in the direction of selection.

Two plausible explanations of these results failed to stand up to tests. Natural selection was excluded because, when artificial selection was relaxed, fitness did not improve. Maternal effects were excluded because hybrids reared by high line mothers had identical lactations to their reciprocals reared by low line mothers. In default of these mechanisms the results remain unexplained.

AN EFFECT OF THE UTERINE ENVIRONMENT UPON SKELETAL MORPHOLOGY IN THE MOUSE

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Maternal effects upon body size have long been known in horse-donkey hybridisation, and more recently demonstrated in Walton and Hammond's classical Shire-Shetland crosses. Green and Russell observed a maternal effect in mice involving the axial skeleton. Reciprocal hybrids between two inbred strains of mice, having predominantly five and six lumbar vertebræ respectively, showed a difference in number of lumbar vertebræ in the direction of the maternal strain. We have confirmed this finding, and have shown by means of egg transfer that the maternal effect is mediated through the uterine environment provided by the mother rather than through the egg cytoplasm. Number of lumbar vertebræ is not related to body size, hence the influence exerted by the uterine environment is presumably not a simple nutritional one.

THE SPREAD OF GENES IN A CLOSED POULTRY FLOCK UNDER SELECTION

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It has been said that a fit individual will leave descendants many generations after its death. Fitness in this sense is the result of natural selection while in a selective programme fitness is also attached to the character under selection. The spread or decrease of an ancestor's genes may be detected by pedigree studies for a few generations after which changes still occur due to selection but these cannot be detected from pedigrees.

It is possible to partition these changes in the frequency of ancestors' genes into changes due to natural and artificial selection.

The results of such a pedigree analysis in a flock of domestic poultry under selection for egg production led to the conclusion that the response to selection may have been improved if assortative mating had been practised in conjunction with selection.

HYPOPHOSPHATASIA, EXCRETION OF ETHANOLAMINE PHOSPHATE IN CLINICALLY NORMAL RELATIVES

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Hypophosphatasia is a rare osteodystrophic disorder. Biochemically it is characterised by a marked reduction in the level of serum alkaline phosphatase activity and a greatly increased excretion of ethanolamine phosphate in the urine.

The families of 14 individuals presenting with this condition have been investigated. No instance of parental consanguinity was observed. Out of 13 sibs of the propositi, two were diagnosed as having hypophosphatasia. No cases of the disease were found among other close relatives. Slight but definitely abnormal levels of ethanolamine phosphate excretion were observed in the urine of some members of the families. It is suggested that the clinically affected individuals are homozygous for a rare gene which in the heterozygotes leads only to a minor biochemical abnormality. If so, about 60 per cent. of the presumed heterozygotes can be detected by the chromatographic methods used.

The severity of the disease and the age at which the osteodystrophy becomes apparent are very variable. In our own, and in published material, sibs resemble each other rather closely in these respects. This suggests that more than one gene may be involved. Further heterogeneity in the material is revealed by the fact that the heterozygous manifestation rate is different in different sibships.

VARIATION IN THE J CHARACTER OF CATTLE BLOOD

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The J character is a well known antibody-antigen system in cattle. Blood samples from a population of twin cattle have been tested for both J antigen and J antibody, the presence of the one confirming the absence of the other.

Variation in J antibody titre in time occurs, but the causes are not known. Twin studies show that the antibody first appears at about six months of age (except perhaps when transmitted in colostrum) and the titre is highest in autumn. Titres are commonly depressed about the time of calving.

J antigen strengths vary between individuals, but are identical within sets of one egg twins. Among adults, the order or rank of antigen strengths remains constant. Both antigen and antibody are independent of erythrocyte mosaicism

and therefore the J antigen has a value in twin diagnosis. With the aid of the knowledge that the J antigen varies genetically in strength, it is now possible to demonstrate sometimes a genetic difference between twins that are both J positive.

THE LINEAR GROWTH PROCESS IN TWIN CATTLE

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Growth is studied in two populations of twin cattle belonging to the Animal Breeding Research Organisation ; one population is comprised of 60 monozygous twin pairs, and the other of 60 dizygous pairs.

The data consist of regular observations up to an age of 2 years on several linear body measurements.

A study of the population growth curves suggests a simple stochastic representation for the linear growth process.

Variation and co-variation in the linear growth of twin and co-twin are analysed in the light of this hypothetical process.

A large part of the variances and co-variances within twin pairs are explained in terms of a population growth constant.

RYE-WHEAT CHROMOSOME ADDITION AND SUBSTITUTION LINES

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Characteristics of rye, *Secale cereale*, cannot be transferred to wheat, *Triticum vulgare*, by normal breeding methods because there is no meiotic pairing between the chromosomes of the two species in interspecific hybrids and amphidiploids. Therefore to introduce rye characters into wheat, in balanced combinations, with the least disturbance of the wheat phenotype, single pairs of rye chromosomes have been added to the full chromosome set of wheat. Separate single-pair addition lines for five of the seven different rye chromosomes have been obtained by backcrossing and selfing derivatives of wheat-rye hybrids and amphidiploids. Each rye chromosome produces a distinctive modification of the phenotype of wheat, and useful characteristics such as disease resistance, are introduced with some chromosomes. Addition lines differ in fertility and stability.

Lines have also been developed which have telocentric derivatives of rye chromosomes, either as monosomic, or disomic additions. These lines permit the extension of the genetic information obtained from whole chromosome additions.

In the derivatives of crosses of disomic addition lines with wheat monosomics, lines have been extracted in which a wheat chromosome pair has been replaced by a rye pair ; the chromosome number remaining at that of wheat. The rye pair adequately compensates for the absence of the wheat pair, in the few lines so far examined, and the distinctive phenotype of the addition lines is largely retained.

CYTOGENETIC STUDIES WITH ANEUPLOIDS IN COMMON WHEAT

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Genetic analyses of qualitative and quantitative characters in wheat have been conducted with aneuploids and special lines derived from them.

The study of nullisomics and monosomics in Chinese Spring wheat and of F_1, F_2 and F_3 progeny from crosses between these lines and varieties with contrasting

characters has resulted in identifying the chromosomes that carry genes for awning, growth habit, seed coat colour, leaf rust reaction, stem rust reaction, spike density, etc. Changed character expression of F_1 in some instances, and F_2+F_3 ratios modified by characteristic univalent chromosome transmission and breeding behaviour, revealed the critical chromosome(s).

Substitution lines, that is 21 lines in which the 21 chromosomes of a variety are each replaced by the homologue from a donor variety have been used to study disease reaction, awning, earliness, lodging resistance, height yield, etc. Since each substitution chromosome is present in the genetic background of the recipient variety, and since these studies can be conducted in well replicated field trials, the genetic effect of each chromosome on the character studied is clearly expressed and can be statistically measured.

Examples to illustrate these situations are presented and a brief account of new studies is given.

GENETIC VARIATIONS IN THE RESISTANCE OF SHEEP TO INFESTATION WITH ROUNDWORMS

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Nematode worms of various genera inhabit particular regions of the sheep's intestine, and cause damage which forms an important economic problem. The fæcal egg count, to which worms of several genera contribute eggs of identical appearance, is a widely used index of the level of infection, and the only one available from the live sheep. In hill sheep, this index generally rises sharply to a peak in the spring, following the poor winter diet, and falls off in summer and autumn as the condition of the hosts improves. This indicates that environmental conditions have a marked effect on the fæcal egg count, and also that sheep can build up a considerable degree of immunity to infection, which is impaired by malnutrition. The paper will describe an attempt to disentangle some of the factors contributing to the phenotypic variance in egg count, and to determine whether there are appreciable genetic variations in the ability to resist infection.

A SUPPRESSOR OF CITRULLINE DEPENDENCE IN *NEUROSPORA*: A CASE OF SINGLE GENE HETEROSIS

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A citrulline-dependent strain (33442, or *cit*) of *Neurospora crassa* has been used to study reversion to independence. In such mutation experiments plating is done on media supplemented with lysine and canavanine to eliminate leakage. In one experiment where plates were supplemented also with hydrolysed RNA, mutant colonies which were citrulline-independent but pyrimidine-dependent were found unexpectedly. They probably stemmed from one spontaneous mutant clone.

Both *cit* and the pyrimidine requirement are closely linked to the colonial marker *co*, yet the two requirements are not allelic. Crossing *co* pyrimidine-dependent with *co*+ prototroph, most of the *co* pyrimidine-independent segregants are citrulline requirers. This suggests a map order : *co-cit-pyr* (*pyr* could be allelic to the previously known pyr_3), where *pyr* suppresses the *cit* phenotype. It is not possible at this stage to discriminate between suppression due to *pyr* taking over the synthesis of citrulline, or due to less direct metabolic interactions as have been suggested by Mitchell and Mitchell for other interrelations between pyrimidine and arginine mutants.

The *cit* and the *cit*, *pyr* nuclei combine to make a heterokaryon able to grow on minimal medium. This is an example of overdominance, since both nuclei differ by only one mutation.

HYBRID VIGOUR OF F_1 MICE AT LOW ENVIRONMENTAL TEMPERATURE

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Mice of strains A2G and C57BL, and first generation hybrids from these strains, have been bred at two environmental temperatures : $-3°$ C. and $21°$ C. (controls). Fertility was higher in the hybrids than in either of the parental strains. The difference was greater at the low temperature : for instance, the mean number of young weaned by hybrid pairs was about twice as great as by inbred pairs at $21°$ C., about five times at $-3°$ C. Heterosis was shown also in the weights of the young of the hybrid pairs. Variability, especially in breeding performance at $-3°$ C., was much lower in the hybrid than in the inbred mice. Young hybrid mice, transferred from $21°$ C. to $-3°$ C. were more resistant to the cold than young inbred mice.

EFFECTS OF TEMPERATURE, AND OF SELECTING RARE CROSS-OVERS, ON RECOMBINATION IN *ASPERGILLUS NIDULANS*

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When recombination frequencies are measured in that small proportion of all meiotic products in which there has been crossing-over between two very closely linked markers, it was shown by Pritchard in *Aspergillus* and by Doermann in bacteriophage, that recombination in regions adjacent to the selective markers is higher than in the whole population. I have now found, in addition, that when rare cross-overs are selected recombination in a region on the same chromosome as, but not adjacent to, the selective markers may or may not be increased. The occurrence or otherwise of this increase depends on the positions where crossing-over occurs in the chromosome, a distribution which varies with temperature (cf. *Drosophila*). There is no evidence of an effect of this kind when recombination is measured in different chromosomes from that carrying the selective markers.

FURTHER EFFECTS OF DISRUPTIVE SELECTION

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Since the previous report it has been confirmed that lines exposed to Disruptive selection possess considerable free genetic variation and that the line maintained under Stabilising selection (Mather's terminology) possesses much less. In addition, the line maintained under disruptive selection with positive assortative mating has produced striking results. This line has two components in different cultures, one with parents selected for high chaeta-number and the other with parents selected for low, but in each generation all offspring are produced by hybridising the two components. There is in principle maximal (50 per cent.) gene-flow between the two components. (Random-mating would involve only 25 per cent. gene-flow.) Despite this maximal gene-flow, selection now maintains a consistent significant difference between the two components, though at first it did not. The population may therefore be said to have become essentially polymorphic (though not polymorphic by Ford's strict definition), and thus demonstrates that Mather was right in expecting disruptive selection to have polymorphism as one of its results. At the same time, considered as two sub-populations, the line shows that isolation is *not* a prerequisite for divergence. Twenty-five per cent. gene-flow is the maximum to be expected in nature and even 50 per cent. permits divergence

under divergent selection pressures. The demonstration that two adjacent populations of a species are genetically different, though it shows they are not one panmictic population, is therefore not unequivocal evidence that there is an isolation barrier between them.

GENETICS OF SEED GERMINATION DATE IN *PRIMULA VULGARIS*

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During some experimental work involving the germination within one greenhouse of seeds from sixteen different populations of primroses, it was noticed that there were striking and fairly consistent differences between the mean germination dates of seed from different populations, and in some cases between those of seed from different plants in a single population. These differences have now been shown to be controlled by nuclear genes, although the number involved is not known. The differences relate to temperature rather than to date ; it is suggested that if, in natural conditions, there is an optimum date of germination common to all populations in an area, then if the optimum is generally achieved seed from the colder populations must be able to germinate at a lower temperature ; with seed from the different populations sown and kept under identical conditions within one greenhouse, as spring temperatures rise the seed from the colder populations will be the earliest to germinate. The results obtained tend to support this view. The ecological and evolutionary significance will be discussed.

STATISTICAL PROBLEMS IN THE SELECTION OF CROP VARIETIES

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When plant breeders have produced a large number of new potential varieties of a crop, the best of these must be selected by field trial, rejecting those that have undesirable characteristics or low yields and preserving the highest yielders. Often such selection will be conducted in several stages, each stage consisting of field trials for one or more years on the basis of which some varieties can be rejected and others passed forward to the next stage. Questions then arise as to the optimal intensities of selection in different stages, the optimal division of available land or other measure of experimental resources between the stages, and even the optimal number of varieties with which to start and the optimal number of stages, in relation to the total increase in yield achieved. Statistical theory has been used to examine these questions for one relatively simple situation, and has suggested some simple rules as approximations to the best possible. The problem of relating the selection programme to the whole agricultural economy has also been discussed, one tentative conclusion being that a small number of stages and consequently a rapid testing and output of new varieties may often be preferable to more thorough and slower testing.

AN INVESTIGATION INTO THE GENETIC CONTROL OF YIELD IN SPRING WHEATS

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A 4×4 diallel set of spring wheat crosses was analysed in F_1 and F_2 by the method of Jinks and Hayman. The characters used were yield per plant and four components of yield, *viz.* weight per grain, number of grains per spikelet, number of spikelets

per ear, and number of ears per plant. The genetic control of the four components was found to be mainly by additive genes with very little evidence of dominance. There is a very slight indication of gene interaction in two of the components. Yield per plant showed strong evidence of gene interaction.

The use of this set of crosses to test the possibilities of this analysis as a tool in plant breeding will be discussed.

THE GENETIC STRUCTURE OF RYEGRASS STRAINS AND ITS IMPLICATIONS FOR HERBAGE PLANT BREEDING

J. P. COOPER

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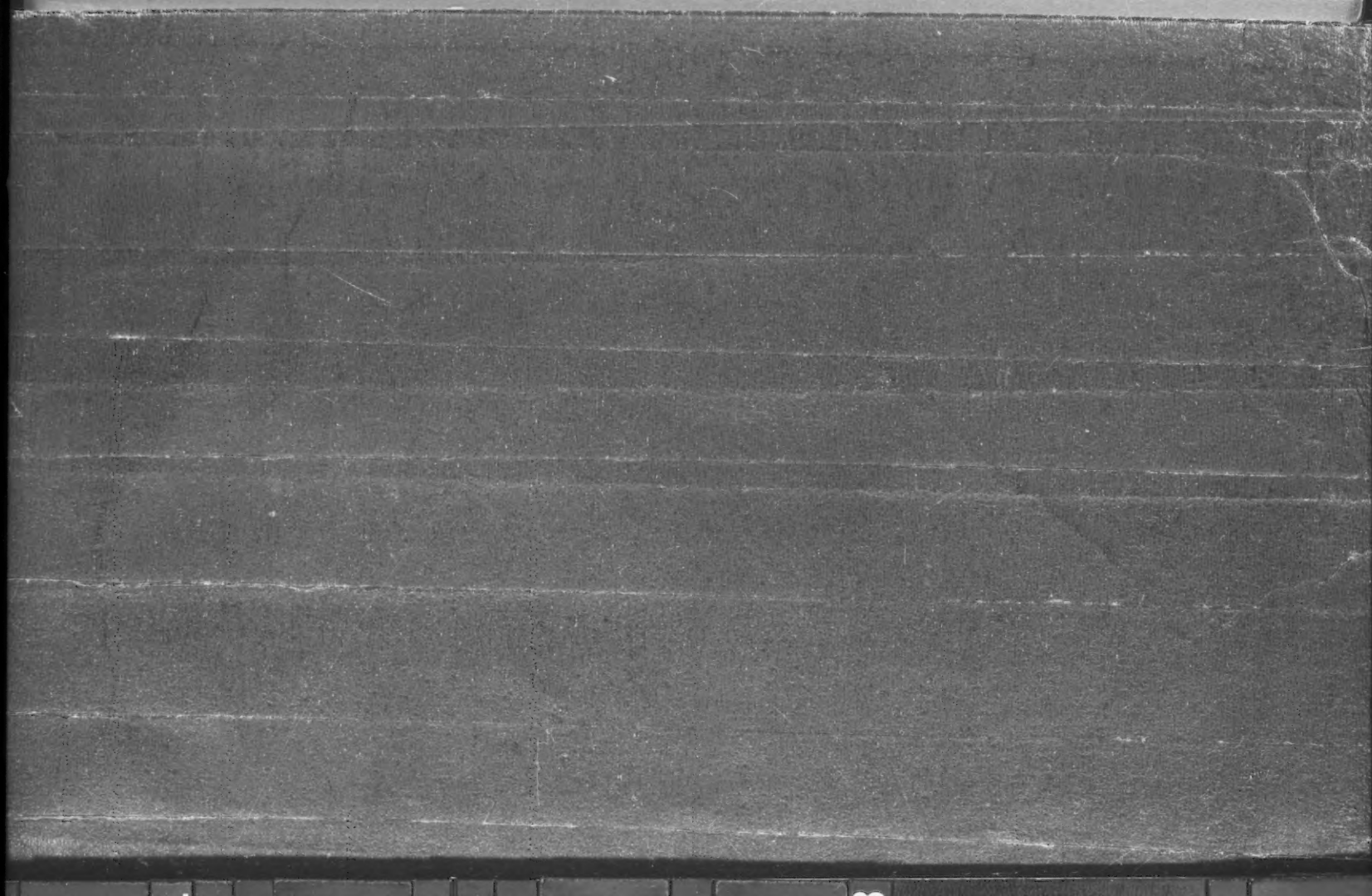
A difficulty in the improvement of many outbreeding crops is the heterozygosity of parent plants and the consequent release of variation during seed multiplication.

A survey has therefore been made of the amount of potential variation within two populations of perennial ryegrass (Irish and Kent) and of its rate of release under selection. The main character studied was date of ear emergence, which is highly heritable, and also shows good " repeatability " from year to year. Early and late selection lines were established, using different initial population sizes, and different intensities of inbreeding.

The results of the first three generations of selection are now available. Considerable response has occurred, even in the lines derived from a single individual, and the means of some of the extreme lines are well outside the range of the original populations. The use of four plants as the basis of selection in each generation has proved sufficient to avoid inbreeding depression.

Considerable potential genetic variation can evidently be carried within a small group of plants of similar phenotype, and rapidly released within a few generations. The implications of such a genetic structure in planning methods of selection and seed multiplication are discussed.



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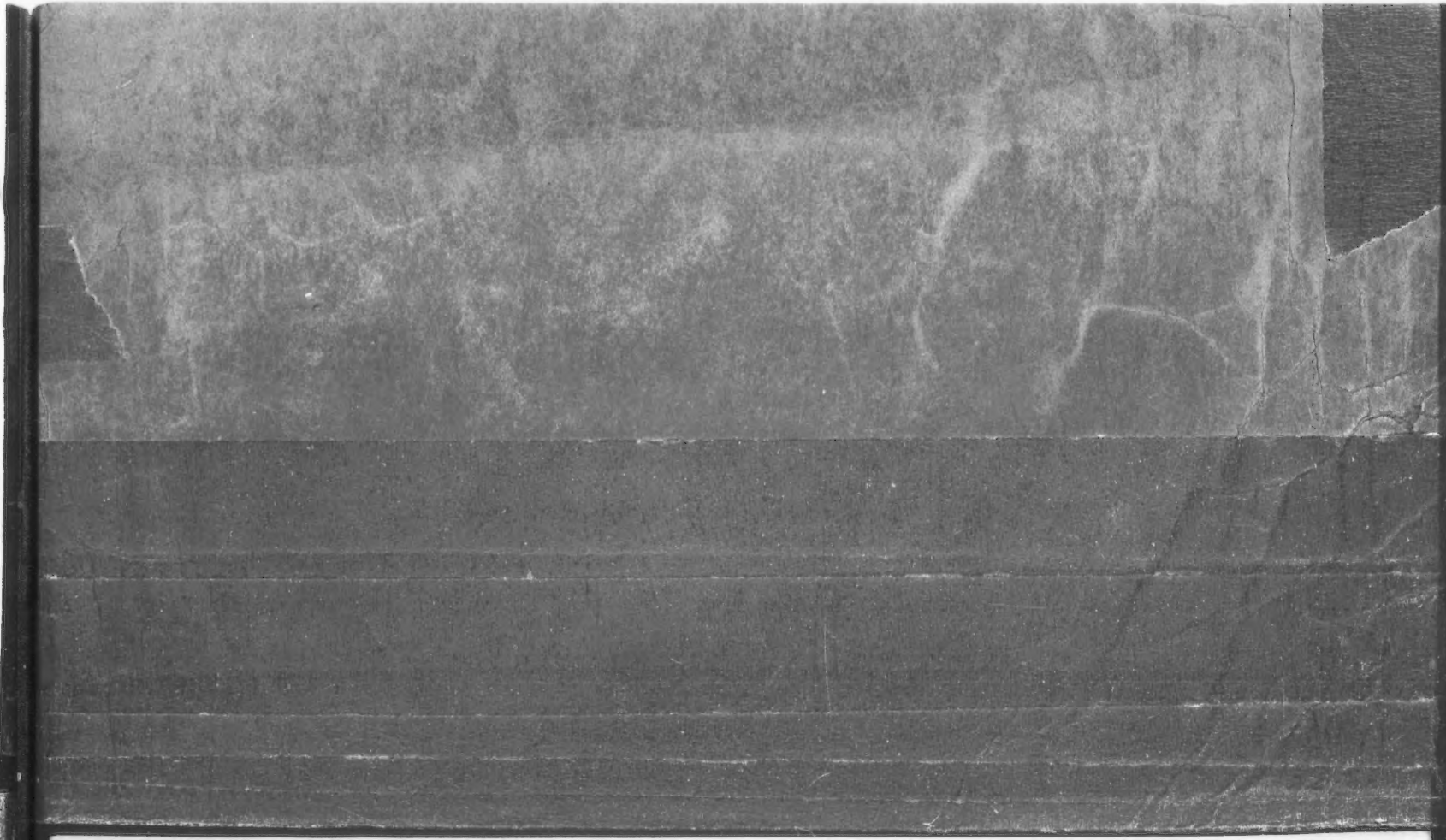
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